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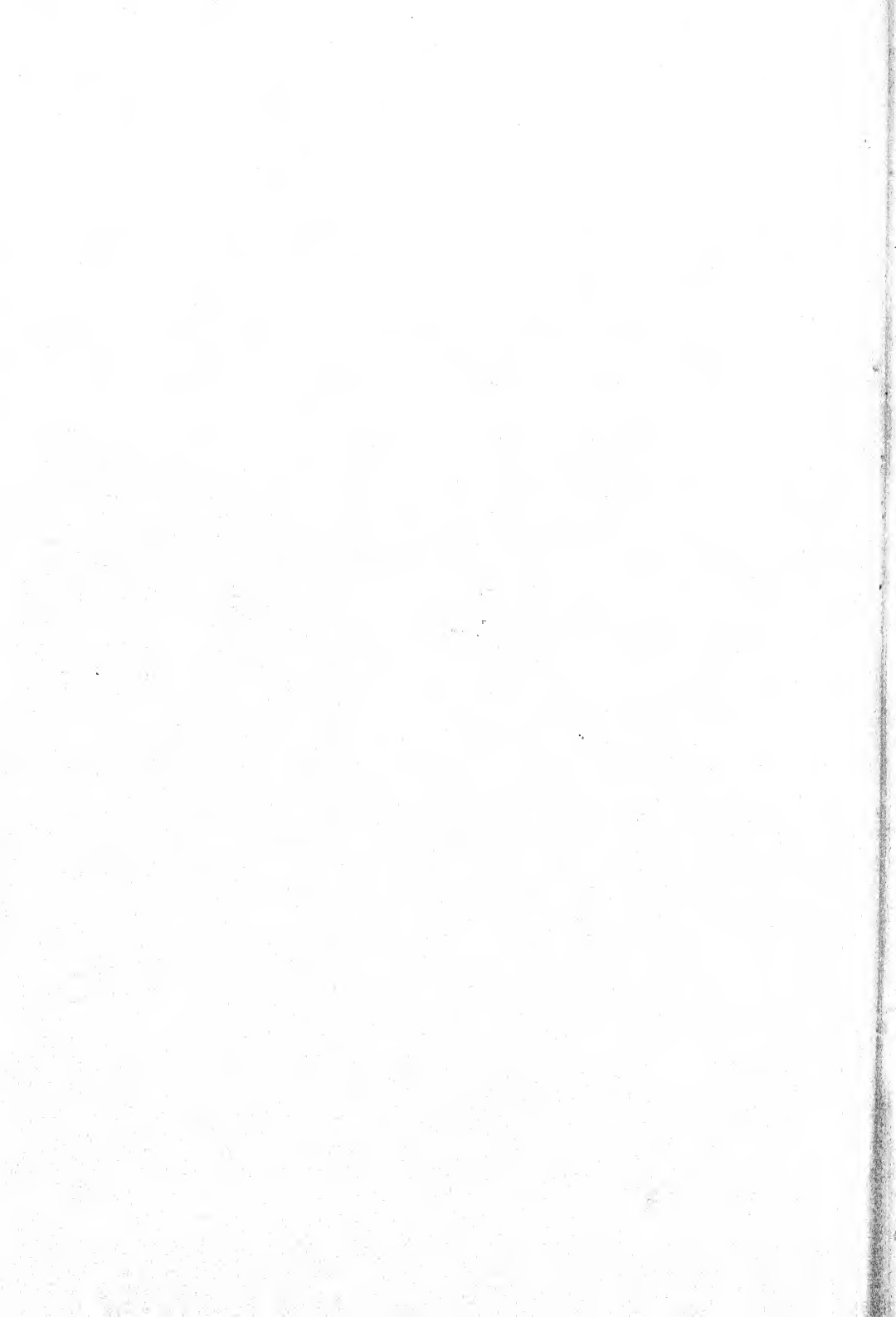
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# PHYTOPATHOLOGY

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## VARIABILITY IN THE FIRE-BLIGHT ORGANISM, *ERWINIA AMYLOVORA*<sup>1</sup>

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Following the discovery of *Erwinia amylovora* (Burrill) Comm. S.A.B., the cause of fire blight, the pathogen was studied culturally by a number of investigators. Among these there appears to be a disagreement as to certain major and minor characteristics of the organism, although methods and media used were apparently identical in many instances. Variation among isolates of the organism has been touched upon by various workers (29, 45, 46) and studied more specifically by a few (27, 41) without, however, their having reached a final conclusion as to the degree or cause of variation.

In an attempt to find a reasonable explanation for differences among cultures reported by others or observed by the writer, 10 different isolates of *Erwinia amylovora* were selected representing 6 different localities and 8 different hosts, and these, after being single-celled by the method of Avery and Leland (5), were studied under different standard and special laboratory conditions. The source of the isolates is shown in table 1.

### MORPHOLOGY

*Size of Cells.* The isolates showed considerable variation in behavior on various laboratory media. Bacteria were found to vary in size regardless of whether taken from the infected parts direct or from cultures. To study variation in size the organisms were passed by inoculation through a pear shoot 3 times, reisolated, and then grown for 24 hours at 28° C. on nutrient meat-extract agar adjusted to pH 6.9. Smears were made from the water of condensation in an agar tube and stained by Gram's method. Five

<sup>1</sup> This is abridged from a thesis presented to the Graduate Division of the University of California in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

The writer wishes to express his appreciation to Dr. H. Earl Thomas and Prof. Ralph E. Smith for their advice during the investigation and for aid in preparation of the manuscript.

Contribution from the Division of Plant Pathology, University of California, Berkeley, California.

TABLE 1.—Source of isolates<sup>a</sup> of *Erwinia amylovora*

Laboratory number	Isolated from	Part of the plant	Date of isolation	Geographical locality
50	<i>Pyracantha angustifolia</i>	Stem (canker)	Aug. 28, 1929	Berkeley, California
55	<i>P. angustifolia</i>	Berry	Jan. 4, 1932	Berkeley, California
57	<i>P. coccinea lalandi</i>	Blossoms	May 25, 1930	San Jose, California
64	<i>Photinia arbutifolia</i>	Blossoms	Sept. 10, 1931	Berkeley, California
77S <sup>b</sup>	<i>Pyrus malus</i>		Sept., 1915	Unknown
500-B	<i>Crataegus monogyna</i>	Twig	July, 1931	Ithaca, New York
501	<i>Crataegus oxyacantha</i>	Twig	May, 1931	Ithaca, New York
507	<i>Crataegus crus-galii</i>	Twig	July, 1931	Ithaca, New York
“Noble”	<i>Pyrus communis</i> Bartlett	Root	Jan. 4, 1932	Walnut Grove, Calif.
SC	<i>Pyrus communis</i>	Twig	June, 1925	South Carolina

<sup>a</sup> In this paper the word strain is used as synonymous with the isolate.

<sup>b</sup> This culture, isolated by E. F. Smith, was received from the American Type Culture Collection Bureau under the number 493.

hundred individuals were used to compute the mean size of the cells of each isolate. In the present work only the length of the organism was considered. Measurements were made by the use of the Spencer filar micrometer.

According to W. L. Trotzky (52), who studied variation and significance of length in *Bacillus typhosus* and *B. dysenteriae*, the length of bacteria may be considered as an individual and constant characteristic of each strain. Strains that were nearly identical in morphological, biological, and serological properties very often were characterized by the fact that the length of the bacterial cells seemed to be very constant and to be hereditarily transmitted.

Using the method outlined above, the writer obtained the following data on length of the different isolates. Isolate 501—0.93  $\mu$ ; isolate 64—1.04  $\mu$ ; isolate 507—1.09  $\mu$ ; isolate 55—1.30  $\mu$ ; isolate “Noble”—1.30  $\mu$ ; isolate 57—1.38  $\mu$ ; isolate 500-B—1.40  $\mu$ ; isolate 50—1.50  $\mu$ ; isolate SC—1.50  $\mu$ ; isolate 77S—1.71  $\mu$ ; isolate R (rough form of culture)—1.63  $\mu$ . To find how significant these measurements were, isolate 64 was mixed with isolates 50 and 77S and, after 3 successive passages at 24-hour intervals in nutrient broth of pH 6.9, the mixed culture was plated out on nutrient agar. After 48 hours of incubation at 28° C. several transfers of single colonies were made on slants of nutrient agar and kept for 2 weeks at room tempera-

ture after a preliminary incubation of 24 hours at 28° C. Subsequently, the cultures were transferred to slants of nutrient agar and 24 hours later smears were made as in the first experiment. The difference in length was so evident that there was no doubt that the cultures represented different isolates. The arithmetical means obtained for cultures used in this last experiment were: 1.07  $\mu$ , 1.47  $\mu$ , 1.68  $\mu$ . The mean in each case represented the average of 200 individuals for each culture.

It is interesting to note that the magnitude of length was in every case greater for isolates that proved to be slightly or very slightly virulent. There exists no correlation between the host from which the isolate came and the length of the organism.

*Size and Form of Colonies.* Ordinary nutrient agar did not depict strikingly the difference in size and shape of colonies between different isolates of *Erwinia amylovora*. However, if to this agar there was added 5 per cent commercial sucrose, characteristic features of differential value could be easily observed (Table 2).

TABLE 2.—*Colony characteristics of ten isolates studied*

Isolate	Description of colonies on 5 per cent sucrose nutrient beef-extract agar
50	Very slow grower. Colonies visible after 48 hours' incubation at 28° C. Colonies small, convex, entire, compact, granular.
77S	Grow very slowly. Colonies visible after 48 hours' incubation at 28° C.
SC	Colonies small, lobate.
55	Colonies small after 24 hours. After 48 hours' incubation at 28° C.
57	Colonies large, round, shiny, moist with the edges thin and very transparent.
64	The growth has tendency to spread so that in a few days there are very
501	many coalescent colonies.
507	
Noble	Slightly smaller than 55.
500-B	Like 55 but erose.

All isolates were grown also in 10 per cent sucrose nutrient agar. The characteristics of the colonies approached those on 5 per cent sucrose nutrient agar, but, after 5 to 7 days, there appeared a peculiar translucent outgrowth from the margin of the colony. This growth was of jelly-like consistency and, upon direct microscopic examination, was found to contain living bacilli. When small portions of this growth were transferred to nutrient beef broth of pH 6.9, there could be observed no growth in many tubes thus inoculated. There was a striking slowness in the appearance of colonies on plates and, again, some plates showed no growth of any kind, even after 20 days of incubation. Such colonies, on a nutrient agar plate,

were very small, translucent, slightly convex, and white and shiny by reflected light. The growth dies out on the plate within a week. This phenomenon was rather common for each isolate studied on 10 per cent sucrose nutrient agar. The pathogenicity and other properties of this type of *Erwinia amylovora* will be discussed later.

It already has been pointed out that the different isolates showed different rates of growth on the same media. To obtain an idea of the relative colony size of each of the isolates, they were inoculated into nutrient broth of pH 6.9 and transferred daily for 3 consecutive days. After this time plates were poured using nutrient beef-extract agar of pH 6.9 and incubated for 48 hours when the measurements of colonies were made. Each value for the size of colony of an isolate is represented in table 3 by an arithmetical mean of 250 individual colonies.

TABLE 3.—Arithmetical means of colony size of 10 isolates of *Erwinia amylovora*

Isolate No.	64	Noble	507	SC	57	50	501	55	550-B	77S
Mean colony diameter in microns ...	1073.6	644.2	568.5	550.5	532.8	516.5	494.6	402.5	389.3	355.2

It will be seen that there is a tendency for more pathogenic isolates to form larger colonies, excepting the large-size rough colonies to be mentioned later.

Eosin-methylene blue and Endo media were tried for differentiation of strains of *Erwinia amylovora*. The Difco brand of these media was used. Eosine-methylene blue medium shows rather distinctly the difference between strongly and weakly pathogenic strains. The less virulent strains always show a metallic lustre, absent in strongly virulent cultures. The slanted medium appears to be better for practical purposes, as it gives more rapid growth and is better for reading results. On Endo agar plates the growth for all the isolates studied was alike. No important diagnostic value can be assigned to this medium because of the sensitivity of the medium to light and consequent difficulty in reading the colors.

Lithium chloride agar<sup>2</sup> gave a rather sharp differentiation of weak and strong isolates. In this medium isolates 50, 77S, and SC appeared as very flat, translucent, spreading; while the rest of the isolates were raised, not spreading, and more or less opaque. No involution forms were observed on this medium.

<sup>2</sup> MgSO<sub>4</sub>, 3 gms.; K<sub>2</sub>HPO<sub>4</sub>, 2 gms.; (NH<sub>4</sub>)<sub>2</sub>PO<sub>4</sub>, 6 gms.; lithium chloride, 5 gms.; sucrose, 20 gms.; distilled H<sub>2</sub>O, 1000 cc., agar 20 gms. Medium adjusted to pH 6.9.



*Motility.* Stained by the Casares-Gil method, the organism was found to be motile by peritrichiate flagella, as shown by earlier workers (9). No difference in motility was observed between the different isolates. Arthur's (4) observations as to the organism being more sluggish in old cankers than in very recent infections could not be substantiated. The organism seemed to be equally motile, irrespective of the stage of the infection.

#### PHYSIOLOGY

To check previous work on cultural characteristics of *Erwinia amylovora* and to study the extent of cultural variability, the 10 isolates were used.

*Temperature Relations.* The 10 isolates were grown in nutrient broth and in a synthetic medium.<sup>3</sup> In each case pH was adjusted to 6.9 with  $\frac{n}{10}$  NaOH. Inoculation was made with a 2-mm. loop from the growth on nutrient agar slants incubated at 28° C. for 48 hours. The amount of medium in each test tube was 10 cc. Readings were taken after the first 24 hours. The temperature at which cloudiness of broth was the heaviest was considered to be the optimum. The experiment was repeated 3 times, similar data being obtained in all cases. It was evident that the optimum temperature of growth for most of the isolates studied is 28° C. The isolates that were designated as weakly pathogenic are slower in growth than those that are strongly virulent. It is important to note that some isolates grow fairly well at low temperatures, such as 15° C. and 19° C.

To determine the temperature at which *Erwinia amylovora* completely terminates growth and multiplication, the same tubes were used as for the determination of optimum temperature. Tubes were kept longer at low and high temperatures. After a week's incubation it was found that at 3° C. there was no perceptible growth in any tube; at 8° isolates 64, 501, and 57 showed fairly good growth; at 12° all the tubes were cloudy; at 31° and 34° all the tubes showed fair growth. No growth was observed in tubes incubated in 37° and 40° C. chambers. Isolates that showed no growth at 3° and 8° were plated out and the plates with original tubes placed at 28° C. Tubes were cloudy in 24 hours and colonies were visible on plates after 36 hours. The organism may remain viable at 3° C. in nutrient broth as long as 3 months without any transfer to fresh medium.

*Death Point.* The upper thermal death point for *Erwinia amylovora* has been placed at 43.7° to 49° C. Two thermal death-point tests were made, employing quadruplicate cultures in each trial. Trials were made at the following temperatures: 45.1°, 48.3°, and 49.5°. The trials were

<sup>3</sup> The ingredients of the nutrient broth were as follows: Peptone (Difco) 10 gms.; beef extract (Liebig), 3 gms.; NaCl, 5 gms.; distilled water, 100 cc. The composition of the synthetic medium was: MgSO<sub>4</sub>, 0.3 gms.; (NH<sub>4</sub>)<sub>2</sub>PO<sub>4</sub>, 6 gms.; KCl, 2 gms.; glucose, 20 gms.; distilled water, 1000 cc.

made for isolates 64, 50, SC, 500-B, and R, selections being made on the basis of relative pathogenicity of these isolates to pear. The isolates 50, SC and R, failed to grow in broth and agar cultures after 10 minutes' heating at 48.3°, but grew well after the same exposure at a temperature of 45.1°. The isolates 64, SC and 500-B failed to grow in broth and agar cultures after 10 minutes' heating, at 49.5°, but grew well after the same exposure at a temperature of 48.3°. Thus the upper thermal death point for isolates 50, SC, and R is between 45.1° and 48.3°, and for isolates 64 and 500-B, between 48.3° and 49.5° C.

*Hydrogen-ion Concentration.* D. H. Jones (29) reported a neutral reaction as the optimum for *Erwinia amylovora*, while V. B. Stewart (46) found the optimum reaction between 8 and 16 on Fuller's scale, which, converted into pH values by means of the formula of Quirk and Fawcett (42), would give pH 7.4 and pH 6.8, respectively.

To study this point further, nutrient broth and liquid synthetic media<sup>4</sup> were prepared. The pH for each lot of medium ranged from 3 to 10 with intervals of 0.2 of a unit and was checked by both colorimetric and quinhydrone methods. Each isolate was inoculated into 3 tubes of each pH and after 24 hours the reading was taken. It was found that the optimum pH at 28° C. for most isolates is 6.8 or slightly above. Moreover, there was a variation in acid tolerance among the isolates. Isolate 50 is able to grow at pH as low as 4.0, and isolate 64 at pH 4.4, while isolate 507 has the narrowest pH range of all. Most of the isolates grew from pH 4.8 to pH 8.8. These data agree fairly well with those of Pierstorff (41). On the alkaline side all the isolates behaved alike and this is in agreement with Howard's (27) findings. It must be pointed out that the growth in synthetic medium at low hydrogen-ion concentrations was not very prompt. The low pH at which the growth of *Erwinia amylovora* may take place helps to explain why the organism thrives in green pear fruits which have pH range of 4.2-5.36 (40).

*Desiccation.* The influence of such a physical factor as desiccation on the life of *Erwinia amylovora* is no doubt of some importance. L. R. Jones (30) could not recover the organism from a cover glass after 76 hours' drying at room temperatures. D. H. Jones (29) and V. B. Stewart (46) recovered the organism after 6 and 9 days, respectively, of drying on cover glasses at room temperature. Hotson (26) found the organism alive on branches about 4 cm. in diameter, which were dried in the laboratory 39 days after removal from the tree. In exudate from pear fruit, black and hard, the organism was alive after 13 days of direct exposure to sunlight and after 14 days with fruit of Jonathan apple. Thomas (48) has studied

<sup>4</sup> The synthetic medium was composed of MgSO<sub>4</sub> 0.3 gms.; K<sub>2</sub>HPO<sub>4</sub> 2 gms., NaCl 3 gms., asparagin 4 gms., dextrose 10 gms., and distilled water 1000 cc.

the desiccation of *Erw. amylovora* on the surface of the honey frame and on the surface of the comb. He found the organism still alive after 20 days on the frame, and after 50 days on the surface of the comb. Parker,<sup>5</sup> investigating the longevity of *Erw. amylovora* on combs of the beehive, states that the organism "was not recovered from artificially infected combs kept inside the hive through the winter. In the laboratory it survived a relatively short time (less than 17 days) at the higher temperatures and higher humidity reasonably near the conditions obtaining in the hive itself." It is evident that under certain favorable conditions *Erw. amylovora* can survive in a dry state for a very long time. Pierstorff (41) found organisms still viable in the dried bacterial exudate (ooze) that had been kept in the laboratory in a vial for 2 years. The writer was able to isolate viable bacteria from bacterial exudate kept in a vial in a dry laboratory closet for 2 years and 10 months.

To ascertain if different isolates of *Erwinia amylovora* are alike in their reaction to desiccation, 2 different experiments were performed. There were only 4 isolates used in these tests, namely, 64, 500-B, SC, and R. In the first experiment the isolates were grown on beef-extract nutrient agar for 24 hours; suspensions of the growth then were made in 0.85 per cent NaCl solution. Using a 2 mm. platinum loop, one drop of each suspension was smeared over sterile cover glasses so as to form a very thin film. The cover glasses were placed on the vaselined inner surface of the lid of a moist chamber. Different degrees of dryness from 10 to 100 per cent were provided by the use of the Lesage (34) method. Exposure over the surface of water was considered as at 100 per cent relative humidity. One series was included for laboratory air. A control for vaseline also was included. The exposure time for this experiment was as follows: 10, 20, 40, 55, and 60 minutes; 2, 4, 8, 10, 12, 16, 18, 20, 24, and 36 hours. The organisms survived under the conditions of the experiment from 18 to 36 hours. The degree of humidity seemed to be of no significance, as far as the longevity of *Erw. amylovora* is concerned in this experiment. However, culture SC was killed in a shorter time (18 to 20 hours) at all humidities than the rest of the cultures (24 to 36 hours), while culture R was the most resistant culture in the experiment. This property of withstanding adverse conditions by some isolates may perhaps help to explain the awakening of some old and dried up cankers, which, even by some experienced blight men, may be pronounced as extinct.

In the second experiment, nutrient broth, to which strips of gauze 3 inches long and  $\frac{1}{2}$  inch wide were added, was sterilized and inoculated with the same isolates as in experiment one. After 48 hours the gauze strips were

<sup>5</sup> Parker, K. G. The fire-blight disease (*Erwinia amylovora* (Burr.) Comm. S.A.B.): Overwintering, dissemination and control. (In press.)

removed and suspended in large sterile test tubes kept at room temperature. Pieces of these strips were cut off and cultured from time to time. This experiment showed that isolates 64 and 500-B were not viable after 6 days; isolate SC could not be recovered after 4 days, and isolate R was killed after 10 days.

Both experiments were repeated and in each case the same results were obtained. Thus, it is safe to conclude that different degrees of susceptibility to dryness exist among isolates of *Erwinia amylovora* and that the rough type is characterized by greater resistance to adverse dry conditions.

#### CULTURAL CHARACTERS

*Nutrient Beef Broth.* Growth is prompt after 24 hours' incubation at 28° C. Isolates 50, SC, and 77S grow very slowly at pH 6.9, but grow promptly at pH 7 to 7.2. An occasional pellicle in the form of a ring was observed in isolate 507.

*Potato-dextrose Broth (pH 6.9).* Prompt growth after 24 hours' incubation at 28° C. Isolates 50, 77S, and SC grew slowly the first twenty-four hours. Slight sediment was formed by isolates 64, 55, 57, 500-B, 501, 507, and "Noble", and more sediment formed by 50, 77S and SC.

*Beef-extract-Peptone Agar.* Streaks on nutrient agar slants of different hydrogen-ion concentration did not reveal any striking characters. Usually the colony is white, glistening, moist, filiform, butyrous.

The use of 5 per cent sucrose nutrient agar is very valuable in bringing out certain differences between isolates. All isolates grow very luxuriantly on this medium, and differences are most striking after 3 days' incubation at 28° C. On this medium some isolates were characterized by white, translucent and spreading growth. The mass of organisms flows down and collects at the bottom of the slant in a very short time, leaving on the slant a thin translucent film. To this type belonged isolates 64, 501, and 55. Some isolates were characterized by grayish, heavy growth along the streak, raised, echinulate, coarsely granular by transmitted light, butyrous. To this type belonged isolates 500-B, 57 and "Noble". Some isolates were characterized by white, not spreading, beaded growth, with very abundant islands, and butyrous. To this type belonged isolates SC and 507. Other cultures possessed grayish, not spreading, filiform, slightly raised growth, less shiny than in group one, and finely granular by transmitted light. To this type belonged isolates 50, and 77S.

*Gelatin Liquefaction.* Gelatin-liquefaction trials were made, employing Frazier's method (16) for the detection of proteolysis. Also, a synthetic medium of the following composition was used: MgSO<sub>4</sub>, 0.3 gms.; (NH<sub>4</sub>)<sub>2</sub>PO<sub>4</sub>, 6.0 gms.; KCl, 2.0 gms.; glucose, 10 gms.; gelatin, 200 gms.; distilled water, 1000 cc. The reaction of this medium was pH 7.0 after

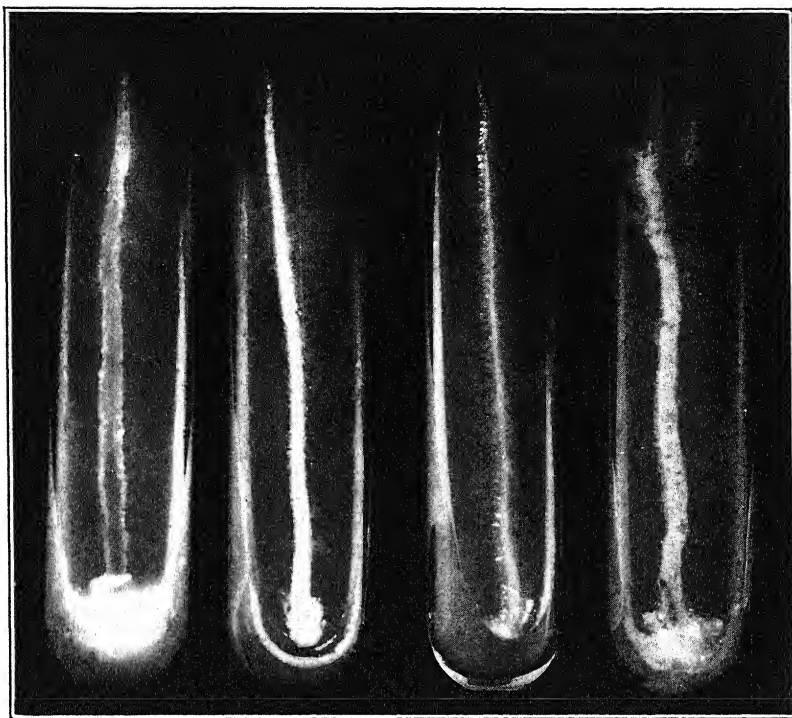


FIG. 1. Isolates of *Erwinia amylovora* on slants of a 5 per cent potato-sucrose-peptone agar. The isolate numbers from left to right are as follows: 64, 50, 500-B, and SC.

sterilization in the autoclave. All of the isolates studied, except 507 and 57, gave positive tests. Gelatin-liquefaction tests indicate proteolytic activities of *Erwinia amylovora* from none to very strong. Pathogenically weak strains show proteolytic activity far greater than that of the most pathogenic strains of the test.

*Indol Production.* None of the isolates of *Erwinia amylovora* produced indol after 20 days in Dunham's solution.

*Nitrate Reduction.* The organisms were grown in nitrate broth for two weeks and tests were made for the presence of nitrites. There was no reduction of nitrates to nitrites by any of the isolates. However, reduction of nitrates to ammonia was found for isolates 64, 55, 57, 500-B, 501, 507, and "Noble", and not for cultures 50, SC, and 77S.

*Action on Milk.* Skimmed milk plus 1 per cent of litmus indicator was distributed in 8 cc. amounts in test tubes, sterilized in the Arnold for 3 consecutive days and then inoculated. For each isolate 3 test tubes were inoculated and incubated at 28° C. for various periods of time. From the results obtained it is evident that the final principal action of the isolates

on milk is gradual digestion of the casein. No striking differences between highly pathogenic and weak strains could be observed, though the weaker strains seemed to decolorize the litmus indicator in a shorter time than the stronger strains.

*Action on Starch.* There was no diastatic action on starch (iodine test) shown by any of the isolates of *Erwinia amylovora*.

*Ammonia Production.* All isolates except 50, 77S, and SC gave a strong positive test for ammonia in nutrient broth after 10 days, using the method of Hansen (21) and Makris (36). Isolates 50, 77S, and SC gave a very slight positive test for  $\text{NH}_3$ .

*Acetyl-methyl-carbinol Production.* The inability of the organism to produce acetyl-methyl-carbinol in peptone-sucrose broth was shown by the Voges-Proskauer (54) test.

*Carbon and Nitrogen Metabolism.* Sugars, Glucosides, Organic acids, and Alcohols.—The organisms were grown in a synthetic medium ( $\text{MgSO}_4$ , 0.3 gms.;  $\text{K}_2\text{HPO}_4$ , 2.0 gms.;  $(\text{NH}_4)_2\text{PO}_4$ , 6.0 gms.;  $\text{H}_2\text{O}$ , 1000 cc.) to which 1 per cent of various carbohydrates, alcohols, and glucosides was added. In order to test the reaction of the medium during bacterial growth, 0.5 cc. of a 1 per cent alcoholic solution of brom cresol purple and 0.4 cc. of a 1 per cent solution of cresol red was added to each liter of medium (11). The medium was sterilized by filtering through a Berkfeld V candle. A small tube with a closed end was inverted in each tube of medium and used in place of the usual fermentation tube to determine the presence of gas and also whether or not the organism would grow anaerobically. The cultures were inoculated in triplicate from a 24-hour-old agar slant and examined at regular intervals of 48 hours.

In table 4 there are presented data on the growth and reactions of the different isolates in sugars and acids. In all sugars utilized by the isolates there was production of acid but no gas. All the isolates used inulin with the production of alkali and dextrin with the production of acid. Of the glucosides, arbutin, phloridzin, salicin, and amygdalin were fermented with production of acid.

*Amino Acids.* Table 4 shows the behavior of the different isolates of *Erwinia amylovora* in synthetic liquid media in which the source of nitrogen was substituted by the corresponding amino acid. Another experiment was made in which the source of both carbon and nitrogen was amino acid. Because the results in both cases were the same, the data are presented together. Of all amino acids tried in the experiments, alanine, leucine, and proline were used by some isolates, while asparagin, which is the amide of aspartic acid, is utilized by all the cultures tested and serves as a source of both nitrogen and carbon.

*Relation of Asparagin to Infection.* It is a general experience that green

TABLE 4.—*Fermentation of sugars, alcohols, glucosides, organic acids and other compounds*

	Not fermented by any isolate	Fermented by all isolates	Fermented by the isolates listed
Carbo- hydrates	Xylose Rhamnose Starch	With production of acid: Arabinose, mannose, glu- cose, fructose, maltose, cellobiose, sucrose, raffinose, dextrin. With production of alkali: Inulin	With production of acid: <i>Galactose</i> 64, 50, 55, 57, 77S, 500-B, 501; <i>Lactose</i> SC, 507, "Noble", 64, 50, 77S, 501.
Gluco- sides		With production of acid: Arbutin, phloridzin, salicin, amygdalin.	
Alco- hols	Dulcitol		With production of acid: <i>Manitol</i> 64, 50, 55, 77S, 500-B; <i>Glycerol</i> 50, 55, 77S, SC, 500-B, 501, 507, "Noble".
Amino- acids	Glycine, valine, iso- leucine, glutamic acid, cystine, cysteine, tyrosine, tryptophane.	With production of alkali: Asparagin	With production of alkali: <i>Alanine</i> 77S, SC; <i>Leucine</i> 50, 77S, SC, 501, 507, "Noble"; <i>Proline</i> 55, 77S, SC, 500-B, 501, "Noble".
Organic acids	Ammonium benzoate, ammonium oxalate, maleic acid, malonic acid, sodium ben- zoate, sodium salicyl- ate, tartaric acid, valeric acid.	With production of alkali: Ammonium citrate, citric acid, hippuric acid, malic acid, sar- cosine, sodium citrate.	With production of alkali: <i>Succinic a.</i> 500-B, 501, 507, "Noble"; <i>Ammonium lactate</i> 64, 50, 55, 77S, SC, 500-B, 507, "Noble".
Miscel- laneous	Melanamide Urea		With production of alkali: <i>Edestin</i> 64, 57, 77S, SC, 501, 507, "Noble".

pear fruits are especially susceptible to "fire-blight". According to analyses at hand (56) the pear fruit contains from 0.42 to 0.52 per cent of asparagin, dry weight. The mature pear fruit contains less than 0.1 per cent of asparagin, dry weight. Disregarding pH of the fruit juice, the value for which remains about the same, according to Overholser (40) and Buxton and Darbishire (10), can the difficulty of obtaining infection on ripe pear fruits be correlated with very low asparagin content of the pear juice?

To gather some idea as to how significant asparagin may be in initiating or spreading infection in susceptible and resistant plants, a series of experiments was made. It is well known that fire-blight infection cannot readily be produced by artificial inoculations in a dormant susceptible plant and only occasionally during the growing season in shoots that have ceased termi-

nal growth. It seemed that if infection could be started in such plants after injecting asparagin, the conclusion as to asparagin having played some rôle could be justified.

In one of the experiments a Bartlett pear tree, growing in a balanced solution, and in a very dormant condition, was inoculated with isolate 64 to which asparagin was added just before inoculation; control shoots were inoculated with isolate 64 without asparagin. At the end of 2 weeks the shoots inoculated with isolate 64 plus asparagin were blighting fairly well, while those of the control showed no infection.

In another experiment there were selected 2 2-year-old Winter Nelis pear seedlings, which had made no growth for a considerable time, and, though they had some leaves on them, the buds were hard and mature. Into one of these seedlings was injected 50 cc. of 1 per cent asparagin solution in water, while another plant was given by the same method 50 cc. of distilled water. Plants were inoculated in dormant buds with a 24-hour-old culture and placed in a warm greenhouse, where they were watered daily. After 3 days the seedling injected with asparagin showed a sizeable drop of ooze, while the control plant was free of any signs of infection, even after 2 weeks. The plant treated with asparagin showed a canker 2 weeks after inoculation.

In still another experiment a very resistant plant of *Cotoneaster frigida*, which had not become infected by inoculation with a virulent culture of *Erwinia amylovora* in a number of trials, was given, through injection, 50 cc. of 1 per cent asparagin and afterwards inoculated with a virulent strain of *Erw. amylovora*. Blight was visible 24 hours after inoculation.

In the course of studies of variability of different isolates of *Erwinia amylovora*, it was interesting to know to what extent asparagin is tolerated by the organism. For this purpose a synthetic medium similar to the one described on page 6 was used. To this medium varying amounts of asparagin (Pfanstiel) were added. In concentrations higher than 6 per cent, asparagin formed crystals in the medium. The experimental data showed that the isolates tolerate as high concentrations of asparagin as 6 per cent and thrive very well, utilizing asparagin as a source of both nitrogen and carbon. The weaker strains grew with their usual slowness.

*Relation of Sugar Concentration to Growth of the Fire-blight Organism.* Considering the readiness with which the organisms grow in glucose and sucrose sugars, it was interesting to know what concentration of each of these sugars would inhibit growth. For this purpose the isolates were studied in a liquid synthetic medium to which varying amounts of glucose and sucrose were added. The medium was sterilized by filtration through Berkefeld V filter candles and tubes were allowed to incubate for a week at 28° C. before inoculations were made. The data indicate the ability of *Erwinia amylovora* to tolerate relatively high concentrations of sucrose and glucose: All isolates



grow in 50 per cent sucrose, isolates SC, 501, and 77S failed to grow in 55 per cent sucrose, isolates 507, 50 and "Noble" grow in 60 per cent sucrose. In glucose all isolates but "Noble" tolerate 14 per cent; isolates 64, 500-B, and 77S tolerate 16 per cent; isolate 507 grows in 20 per cent but not in 22 per cent; isolate 57 grows in 25 per cent but not in 26 per cent, and isolate 50 grows in 28 per cent. Isolate "Noble" tolerates 10 per cent but not 12 per cent.

The tubes that showed no growth in the above tests were cultured in the following manner. One cubic centimeter of the content of the test tube was transferred into sterile nutrient broth and another cubic centimeter portion was smeared on the surface of agar in Petri dishes. This was incubated for a week. In no case was growth obtained.

#### VIRULENCE

Arthur (4) was one of the first investigators of fire blight to attempt to "learn why some varieties suffer more from the disease than others". He made a number of inoculations and cross inoculations into pear, apple, quince, etc. Arthur's work is very suggestive of the existence of differences in the organisms used. Jackson (28) isolated the organism of the fire-blight disease from prune and found it to be more virulent than that from pear. Sackett (43) observed differences in incubation periods of organisms isolated from different plants. Pierstorff (41), employing twelve different isolates of *Erwinia amylovora*, made an attempt to find physiological strains. He was able to observe in some cases distinct differences in the percentage of infected twigs and in the average length of twig blighted.

The writer undertook a comparison of variability in virulence of the 10 isolates used elsewhere in this work. The term virulent as employed in this work designates the power to rapidly spread within the tissues producing death of invaded parts. To judge the relative virulence of isolates the percentage of blighted shoots and average length of twig blighted are taken as criteria. Many inoculations were made in the greenhouse using potted plants. A great number of apple and pear seedling plants were used in inoculations at the University experimental plots in Berkeley and San Jose, California. In some cases the cultures used were single-cell cultures. Inoculations in all cases were made into succulent tender tips with the inoculating needle, using 48-hour-old agar cultures. Readings on infection were made at the end of the third week after inoculation.

Table 5 represents the data on inoculations into seedlings of the Beurre Hardy variety of pear and *Pyrus malus*. It is evident that isolates 50, 77S and SC behave similarly on the same host and are physiologically distinct.

From 53 to 230 inoculations (usually 100 to 150) were made for each isolate on each of the following: *Cotoneaster frigida*, *C. pannosa*, *Photinia*

TABLE 5.—Infection produced by different isolates on seedlings of *Beurre Hardy* pear in the field at San Jose, May–July, 1932, and on seedlings of *Pyrus malus* in Berkeley, July–August, 1932

Isolate	Beurre Hardy pear				<i>Pyrus malus</i> : 100 inoculations per isolate	
	First experiment: 500 inoculations per isolate		Second experiment: 500 inoculations per isolate			
	Per cent infected	Average length of twig blighted (in.)	Per cent infected	Average length of twig blighted (in.)	Per cent infected	Average length of twig blighted (in.)
64	98	4.31	92.0	3.90	100	7.09
50	5.2	2.10	4.4	1.78	0	0
55	91.4	5.02	96.0	3.82	90	7.55
57	61.0	4.42	80.0	4.46	71	3.58
500-B	86.6	5.32	92.8	5.45	83	5.91
501	87.6	4.94	78.8	3.27	92	6.28
507	89.2	5.42	96.4	4.96	100	5.52
77S	11.0	3.26	10.0	3.47	6	1.46
SC	29.6	3.14	26.8	3.25	5	2.00
“Noble”	98.6	4.65	86.0	4.70	100	5.54

*arbutifolia*, *Pyracantha angustifolia*, *P. coccinea lalandi*, *P. gibbsii younnanensis*. The results were similar in the main to those with pear and apple but with certain deviations. With *C. pannosa* and *P. arbutifolia*, which are highly susceptible under the conditions of these tests, isolate SC with the former and isolates 50 and SC with the latter were distinctly less virulent than the remaining isolates. On the other hand, with the comparatively resistant *P. gibbsii younnanensis*, isolate 57 as well as 50, 77S and SC produced no infection while the remaining six isolates blighted from 14 to 45 per cent of the inoculated shoots.

The question was raised as to whether it would be possible to explain the peculiar behavior of isolates 50, 77S, and SC on the basis of prolonged culturing on artificial media. For this purpose some of the recently isolated isolates of *Erwinia amylovora* were used to make inoculations along with certain of the ten original isolates. It was found (table 6) that recently obtained isolates 2150 and 50<sup>1</sup> were as weak as isolates 50, 77S, and SC. On the other hand, such isolates as 64 and 507 which had been grown in the laboratory for about 2 years were among the most virulent and the former is still being used as a standard of high virulence after 4 years in culture.

These results constitute evidence of distinct physiological entities, and while prolonged culturing may account in part for the low virulence of some isolates, the close similarity of cultures 50 and 50<sup>1</sup> (table 6) taken from the

TABLE 6.—*Infection produced by old and new isolates of Erw. amylovora, June to August, 1933*

Isolate	Per cent infected		Isolate	Per cent apple infected: 100 inoculations per culture
	Pear: 250 inoculations	Apple: 100 inoculations		
93 <sup>b</sup>	75	74	64	100
95 <sup>c</sup>	68	76	50	0
2120 <sup>d</sup>	30	96	501 <sup>a</sup>	0
2150 <sup>e</sup>	2	0	507	86
2115 <sup>f</sup>	64	84	77S	8

<sup>a</sup> Isolate 50—From same host as 50, but isolated May, 1933.

<sup>b</sup> " 93—From *Cotoneaster pannosa*, Berkeley, June, 1932.

<sup>c</sup> " 95—*C. salicifolia* " " "

<sup>d</sup> " 2120—Forelle pear " May, 1933.

<sup>e</sup> " 2150—*Pyracantha gibbsii younnanensis*, Berkeley, March, 1933.

<sup>f</sup> " 2115—Spitzenberg apple, Sebastopol, June, 1933.

For source of other isolates see table 1.

same suscept and locality at widely different dates, indicates that weakly virulent strains may arise in nature and persist there for rather long periods.

#### ORIGIN AND REVERSION OF VARIANTS

*Dissociation in Bacteria. Animal Pathogens.* In recent years the morphological variation of microorganisms, especially pathogenic bacteria, has been given considerable attention. The knowledge of this variability has practical significance in the diagnosis of a particular microorganism as well as in the other aspects of practical bacteriology, as for instance, serotherapy. The term dissociation in its more limited sense implies the segregation of two quite distinct bacterial forms which are always present in the parent culture and is associated especially with the idea of regular cycles through which bacteria pass in their life history. An excellent treatment of the whole subject of microbial dissociation is found in Hadley's (20) classical monograph on variation and it is deemed unnecessary to go into a detailed review of the subject at this time.

In 1921, Arkwright (2, 3) described two different variants in the dysentery and Salmonella groups of bacteria, namely, smooth ("S") form and rough ("R") form. Since that time the dissociation phenomenon has been observed and reported for many important bacterial pathogens of man and animals.

*Plant Pathogens.* It is only recently that the phenomenon of microbial dissociation has aroused the interest of phytobacteriologists. Though the

literature on the dissociation in plant pathogenic bacteria is meager, there is enough information to indicate that the phenomenon takes the same general course as with the animal pathogens. Miss Hedges (22) in 1924 and Sharp (44) in 1927 noted and illustrated the rough colonies of *Phytomonas phaseoli sojense*, Hedges. Gardner and Kendrick (17) described and presented a photograph of aberrant (rough) colonies of *Phytomonas vignae*, G. & K., and Link and Hull (35) in 1927 obtained rough strains of *Phyt. citri*, *Phyt. medicaginis*, var. *phaseolicola*, and *Phyt. tumefaciens*. *Phytomonas beticola* was reported to undergo very ready dissociation on nutrient laboratory media and in the host by Brown (8), Elcock (15), and O'Neal (39). In 1931 Stoughton (47) called attention to variants in *Phyt. malvacearum*.

Plant pathogens undergo dissociation on solid media and rough types are pathogenic to the hosts, though in some cases to a very slight extent. In some instances the rough types give more pronounced symptoms than the S form. Elcock (15) noticed that the R form of *Phytomonas beticola* produced overgrowths larger than those from the S type.

*Characters of S, R, and Other Types.* The smooth (S) type forms smooth, round glistening, moist, dome-shape colonies with sharply defined limits. Examined under the microscope the colony appears to be finely granular. In bouillon this type produces uniform cloudiness without pellicle. In physiological solution (0.85 per cent NaCl) the S type produces diffused turbidity without floccules. The colonies of the R type have a very different external and internal appearance. They are generally larger than those of the S type, flat, dry, with irregular margins. In the center of the colony there often is a small knob and the surface is wrinkled. In bouillon this form produces abundant precipitate and pellicle. In physiological solution the R type produces flocculation. The bacteria of S type are motile, those of R type slightly motile or nonmotile.

Besides these two mentioned forms, bacteriologists distinguish another type of colony, intermediate between S and R, and designated by the letter O or sometimes by I.

*Agencies Producing Variants.* Variants can be produced by chemical or immunological means, as pointed out by Arkwright (2, 3), Hadley (20), Hoffstadt (25), Koser (31, 32), Kwaschnina (33), Müller (38), not to mention a score of others. Sometimes these variants appear spontaneously. Among the chief factors concerned in the causation of variants are aging of the culture, changes in temperature, food substances, other chemicals, physical state of medium, and specific immune sera.

*Biological Variations in S and R and Intermediate Types.* The dissociative processes bring about not only morphological differences in microorganisms but also produce biological variations in strains thus produced, such as difference in virulence and behavior in nutrient media, (19, 51,

57, 58). In some cases the rough type, which is avirulent when produced, may become highly virulent upon passage through the animal, as shown by Todd (50). In *Bacillus anthracis* there exists a peculiar situation in that the virulent type is usually rough, while the avirulent form is smooth. Eagles (13), in his study of two strains of haemolytic streptococci, found enhanced virulence in the rough variant of one and lack of virulence in that of the other, and concluded that colony appearance is not a reliable index of virulence. Andrews (1) found more serological specificity in a rough type of haemolytic streptococcus than in a smooth type.

*Reversion of R into S.* It generally is conceded that the R type of bacterial species is stable on common laboratory media, especially solid media. The reversion of the R type into the S is brought about by various methods, and there is no general rule laid down for all species of bacteria. The animal bacterial pathogens frequently are changed from R to S type by the passage of the R type through an experimental animal. There are several instances in which the R type was converted into S by use of agar and liquid media. Thus, Edwards (14) successfully reverted the rough type of *Shigella equirilis* into the smooth form by growing the organism on nutrient agar slants of acid reaction at temperatures above 37° C. Koser and Styron (32) reported the conversion of the rough type into smooth by daily or twice daily transfers of *Bacterium dysentery* in glucose broth. They state that "in some instances S colonies appeared as early as the fifth transfer." Both Sharp (44) and Elcock (15) found the reversion of R type into S occurred during passage through susceptible plants in case of *Phytomonas beticola* and *Phyt. phaseoli sojense*, respectively.

*Dissociation in Erwinia amylovora.* Observing the morphological, cultural, and pathogenetic variability of *Erw. amylovora*, the writer wondered if that could not be explained by the dissociation of the organism. Attempts were made to produce variants, and especially the rough form, by the methods known to bacteriologists. The following account gives the outcome of these studies.

*Effect of Temperature on Dissociation of Erwinia amylovora.* To see if the temperature factor is influential in bringing about changes in type of colony, nutrient beef broth of pH 6.9 and 7.2 was inoculated with the isolates of *Erw. amylovora* mentioned elsewhere in this work. The test tubes were placed in incubators at temperatures 3°, 8°, 12°, 15.5°, 19°, 21°, 25°, 28°, 31°, 35° 37°, and 40° C. Every 24 hours streaks from each test tube were made on nutrient 2 per cent agar plates which subsequently were incubated at 28° C. for 3 days, after which time the examination of the resulting growth was made.

This experiment showed that the dissociative process was operative at temperatures of 12° to 25° C., irrespective of pH. The first rough colonies

were observed after 20 days of incubation. The isolates 50, 77S, and SC were the first to show the complete dissociation into the R type (after 10 days), while the rest presented a mixture of R and S types after the 20th day.

The rough colonies were large, flat, wrinkled, and dull. They were firm when touched with the needle and when suspended in 0.85 per cent NaCl solution formed clumps. In all particulars they agreed with the descriptions of the rough type in other bacteria. When transferred to solid media the rough colonies appeared to be stable after numerous transplants. There was no tendency to revert when grown in common nutrient broth.

*Effect of Aging on Dissociation.* As has been mentioned above, the aging of the culture may result in the production of variants. Broth tubes and dilution plates and streaks on nutrient infusion-broth agar that were stored at room temperature for from 4 to 9 months were used in studying this point. All the cultures after prolonged storage showed an abundance of rough type colonies. Again, in tubes with isolates 50, 77S, and SC only rough colonies were present, while in the rest was a mixture of rough and smooth.

Continuous cultivation of the organisms on solid media, organic and inorganic, in no case leads to the appearance of dissociants. Occasionally, however, some isolates, especially SC and 77S, give rise to colonies with numerous minute papillae on the surface of the colony upon streaking on nutrient agar containing 0.5 per cent lithium chloride (Fig. 2, D). The dilution plates made from these papillae reverted to the normal type of colony.

*Effect of Repeated Transfers in Bouillon.* Another experiment was undertaken to ascertain if rapid transfer in liquid media could be used to cause dissociation. Nutrient broth of pH 6.9 was inoculated with the 10 isolates and transfers made into fresh broth every 18 hours. Tubes were inoculated at 28° C. Each tube, after taking a loopful for inoculation into fresh bouillon, was used for streaking agar plates. This procedure produced dissociants in some cultures. The dissociation was, however, very slow and incomplete. After thirty days isolates 50, 77S, and SC produced 10 per cent roughs and maintained it for 2 months. The experiment was then terminated. The other 7 cultures remained unchanged after 2 months.

*Effect of Peptone and Meat Extract on Dissociation.* It has been reported by Koser and Styron (32) that high concentrations of peptone were effective in bringing about microbial dissociation. With this in mind concentrations of Difco peptone from 1 to 20 per cent were tried. Dissociation took place only in one per cent peptone broth incubated at 25° C. for 20 days. Next, the concentrations of meat extract (Liebig's) were changed, the range used being from 0.3 per cent to 5 per cent. No special enhancement of dissociation was observed, the process taking place in the usual concentration of meat extract and in the same way as described above.

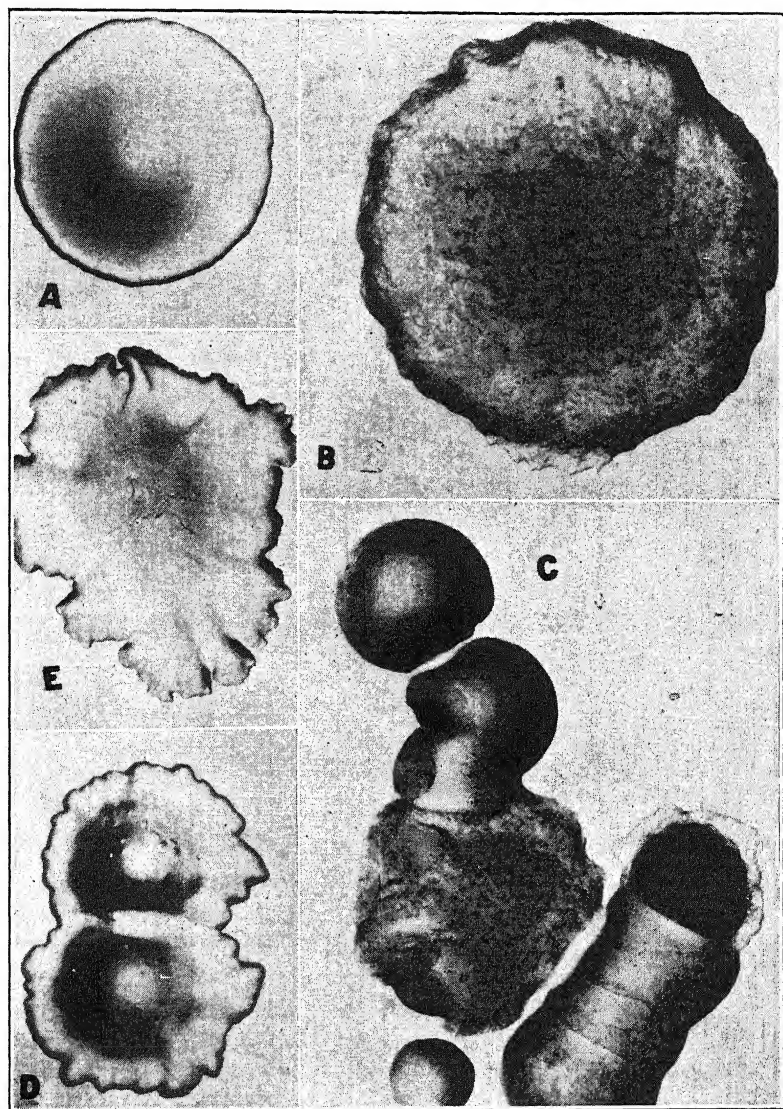


FIG. 2. A. Normal (S) colony of *Erwinia amylovora*. B. Rough type ("R") of *Erw. amylovora*. C. Mixture of rough and smooth colonies of isolate 64. Streak on potato-dextrose-peptone agar plate. D. Secondary daughter colonies on colony of isolate 507. Result of growth on lithium chloride medium. E. Erode colony of isolate 500-B.

*The Effect of Sugars on Dissociation.* Sucrose and glucose were not effective in bringing about dissociation; on the contrary they were found to be important in the reversion process.

*Reversion of R to S.* Since the rough type is stable and cannot be further



dissociated, it was important to gain some information as to conditions necessary to revert the R type into S. Some of the methods of medical bacteriologists were used. The rough type of *Erwinia amylovora* was grown in nutrient broth with pH range from 6 to 8 with intervals of half unit. The temperature range was from 29° to 37° with intervals of 2° C. No conversion of the rough type into smooth was obtained by these methods at the end of 1 month. Immune serum was without effect on the R type. However, when the rough culture was grown in 2 or 5 per cent sucrose nutrient broth, reversion was noticed after 4 to 6 daily transfers. The plating out of the rough culture at this time showed a mixture of R and S types. This conversion could not be observed in 10 per cent sucrose broth. The best glucose concentration for the reversion was found to be 1 per cent in nutrient broth. The effect of sugar on reversion is in agreement with findings of Koser and Styron (32) on dysentery bacteria. This phenomenon of reversion of *Erw. amylovora* from rough to smooth may be important in explaining the epiphytology of fire blight, as can be seen from the data to be presented later.

*Occurrence of R Type in Natural and Artificial Infections.* Does the rough type of *Erwinia amylovora* occur in nature? The answer to this question is, no doubt, significant in clarifying some confusion existing in our knowledge of fire blight. After the laboratory production of the rough type, closer attention was given to colonies appearing on the plate when culturing for the fire-blight organism. The technique used consisted in surface sterilizing the sample, crushing it in a mortar in sterile bouillon, and then making dilution plates, or streaking and smearing on agar plates with glass rods (23, 24).

It was found that the R type may be isolated from old infections, i.e., the infections that are approaching their terminus, as for example old cankers on moderately susceptible trees. In one case a large canker on an apple root was received for isolation. This sample contained varied forms of *Erwinia amylovora*, among which the predominant types were R and O (intermediate). On several occasions old, cracking cankers on trunks of Bartlett pears yielded some rough and intermediate types along with the smooth type. Instances where the rough and smooth were found together in the ooze from cankers also are noted. In all these cases the rough type was very slightly pathogenic for succulent pear shoots and avirulent for shrubs like *Pyracantha angustifolia*. The rough type of *Erw. amylovora* was obtained from old twig infections produced by artificial inoculations into *P. angustifolia*, *P. gibsii younnanensis*, *Cotoneaster frigida* and *Pyracantha* (hybrid). Some samples of blight on *P. angustifolia* and *C. salicifolia* brought from Niles and San Jose, California, yielded a good crop of roughs.

*Attenuation of Erwinia amylovora by 10 Per Cent Sucrose Broth.* In the course of study of the influence of sugar on the behavior of *Erw. amylovora*,



another observation was made that is likely to be of interest in explaining the absence of disease or its very slow course in dry conditions. In studying the sugar tolerance of *Erw. amylovora* the writer noticed the attenuation of the virulent culture 64 when that culture was kept in sucrose synthetic medium of 10 per cent or more of sugar. Succulent shoots of pear seedlings inoculated with a one-week-old culture in 10 per cent sucrose showed a great delay in blighting compared with those inoculated with a culture from 2 per cent sucrose medium. This phenomenon was followed up and a medium containing 10 per cent sucrose was inoculated and daily transfers into fresh tubes of the same medium were made with simultaneous inoculations into soft green shoots of *Pyracantha angustifolia* (Table 7). It is interesting to note the decrease in virulence of almost 50 per cent as a result of the daily transfers in 10 per cent sucrose medium. The organism seemed to have lost some of the power to invade the tissues, but, once it gets into the tissues, it proceeds with the usual speed as indicated by the column on average length of twigs blighted.

*Variants Other than S and R.* Aside from the characteristic R type colonies, a number of variants of *Erwinia amylovora* was encountered during the course of this study. The intermediate type (0) was observed for many isolates and was very stable when kept on solid media as well as in liquid media.

Rhizoid and translucent types of colony were observed infrequently in some isolates. Isolates 507, SC, and 77S sometimes showed rhizoid colonies when plated out from 2- or 3-day-old agar slants. All isolates, when grown on 5 or 10 per cent sucrose nutrient agar, split off very translucent, small, thin, flat colonies that were characterized by a very slow growth on nutrient agar and in nutrient broth. This translucent type of colony had to be transferred at frequent intervals in order to keep the bacteria alive. In cultures 1 week old the bacteria were found dead.

*Virulence of R and Other Variants.* The rough type of *Erwinia amylovora* possesses very slight virulence to green fruits of susceptible varieties of pears and no virulence to the shrubs so far tested, e.g., *Cotoneaster dammeri radicans*, *C. frigida*, *C. pannosa*, *C. salicifolia*, *Photinia arbutifolia*, *Pyracantha angustifolia* and *P. gibbsii younnanensis*. Succulent shoots of apple seedlings were not susceptible to a rough strain of *Erw. amylovora*. The intermediate type of any isolate of *Erw. amylovora* studied was found to be pathogenic to all the above-mentioned hosts, though to a lesser extent than the smooth form.

At this point it is interesting to note that isolate 50, which behaved very much like the intermediate type, was dissociated into S and R types. The resulting R type was found to be avirulent for the shrubs tested and slightly virulent to pear fruits and succulent shoots. The S form of isolate 50 was

3 times as virulent as the parent culture. *Pyracantha angustifolia* was inoculated in the greenhouse with isolate 50 (parent culture) and the corresponding smooth form. Out of 150 inoculated shoots the S form blighted 126, or 84 per cent, while the parent culture blighted only 42, or 28 per cent. The average distance invaded by the smooth form was 1.32 inches; that of the parent culture 0.87 inches.

Isolate 50 produced also the translucent type of colony, which proved to be absolutely avirulent to pear, apple, and other rosaceous susceptibles tested. This translucent type, however, may gradually be changed into a virulent one by repeated transfers into 2 per cent sucrose nutrient broth. Six daily transfers in that medium suffice to make an avirulent translucent culture as virulent as the mother culture. May not this be a factor in bringing about the epidemic of blossom blight when the concentration of sugars in nectaries falls to 2 or 3 per cent during moist weather conditions?

It already has been mentioned that sugars have a pronounced effect on the change of R type into smooth, and from less to greater pathogenicity. It must be said that this change is brought about gradually: the number of smooth-type colonies is gradually increased, and, with this, the virulence is enhanced. De Kruif (12) states that the virulence of a given culture is a function of the proportion of S bacteria present in it and is not due to a general rise or decline of the invasive capacity of each unit in the culture; while Zinsser (59) is of the opinion that organisms contain individuals of different types, and that differences in virulence depend upon the numerical proportions between highly virulent S organisms and the less virulent R types, different environments favoring the preponderance of one or the other. This idea is borne out by the following experiment performed to ascertain the pathogenicity of cultures when passed through 2 per cent sucrose nutrient broth. Isolate 50 (not dissociated) and strain R of isolate 64 produced by enforced dissociation were inoculated into 2 per cent sucrose nutrient broth of pH 6.9 and daily transfers were made into fresh tubes of the same medium. Every day after transfers were made, rapidly growing shoots of *Pyracantha angustifolia* were inoculated in the greenhouse. Table 7 indicates the increase in virulence gained by these isolates as a result of cultivation in sucrose nutrient broth of a low sugar concentration. The isolations of the organisms from blighted plants showed the presence of rough and smooth types up to the 8th passage, while after that only the S type was present in dilution plates made from the diseased tissues.

#### DISCUSSION

The wide fluctuation in the prevalence of fire blight from year to year is well known. The difference of opinion as to the causes of this fluctuation is perhaps equally wide. The principal controversy centers around the

TABLE 7.—*Infection produced on Pyracantha angustifolia in the greenhouse by organisms passed through different sugar concentrations. For each passage one hundred shoots were inoculated with each culture*

Passage	Isolate 50 <sup>a</sup> (Not dissociated)		Isolate 64 <sup>b</sup> (R type)		Isolate 64 <sup>c</sup> (Smooth type)	
	Percentage of shoots blighted	Average length of twig blighted	Percentage of shoots blighted	Average length of twig blighted	Percentage of shoots blighted	Average length of twig blighted
1st .....	30	0.85	0	0	100	4.37
2nd .....	50	1.00	0	0	100	3.94
3rd .....	45	1.17	0	0	100	3.90
4th .....	75	1.15	65	0.43	95	3.66
5th .....	85	1.09	50	0.40	90	3.71
6th .....	90	1.23	67	0.83	90	3.68
7th .....	85	1.30	60	0.60	80	3.80
8th .....	85	1.30	60	0.88	70	3.75
9th .....	85	1.39	95	0.96	65	3.58
10th .....	95	2.01	95	1.58	53	3.50

<sup>a</sup> and <sup>b</sup> Passed through 2 per cent sucrose nutrient broth.

<sup>c</sup> Passed through 10 per cent sucrose in synthetic medium.

mode of dissemination of the organism, with less divergence in view as to the agencies that promote the establishment and development of infection. It has been shown that high atmospheric humidity favors the development of infections already established, and a few authors have taken the view that rain is the chief vector (18, 37, 53). Many investigators, on the other hand, are convinced that insects are the principal agents in dissemination (7, 6, 42, 55).

Some work recently reported from this laboratory (49) together with the matter presented in the foregoing pages may perhaps partly reconcile the divergent hypotheses as to dissemination, and may offer a more exact explanation of the factors governing the inception of infection and its subsequent rate and degree of development.

Dissociation of *Erwinia amylovora*, brought about by such agencies as adverse temperatures, age of the infection, change in abundance or kind of food, may well be expected to take place in nature, thus giving rise to variants of widely different virulence. This is shown to be the case in isolates from old infections of pear and apple and certain shrubs that yielded smooth (virulent), intermediate and rough (less virulent) types.

It was found further that continued culturing of the organism in media containing as high as 10 per cent sugar resulted in attenuation of the organism to the point of slight or no virulence (translucent type). Certain higher sugar concentrations within the range found in nectars, inhibited

growth altogether. On the other hand, repeated transfers in media of low sugar concentration were followed by restoration of the attenuated translucent type culture to the parent type and of rough and intermediate forms to the virulent smooth form. It has been shown by Bentler (6) and earlier workers, and verified in California by Vansell<sup>6</sup> and others (50), that the sugar concentration in the nectaries of blossoms varies widely in inverse relation to the atmospheric humidity, and the volume of nectar undergoes variations of similar magnitude in direct relation with humidity. Thus the nectar of pear may vary from 1 or 2 per cent total sugar in an essentially saturated atmosphere to at least 55 per cent in very dry air. It seems apparent then that rainy or humid weather may profoundly influence the course of fire blight, chiefly in an indirect way, by determining the volume and sugar concentration of the medium that the organism finds in the principal infection court (nectary), which in turn may not only favor the rapid increase of virulent forms of the bacteria but also change the weakly pathogenic forms to the virulent state. A complete hypothesis to account for the development of epiphytotics of blossom blight also would include favorable temperature and intervals of weather conducive to the flight of blossom-visiting insects.

#### SUMMARY

Ten different isolates of *Erwinia amylovora* representing 6 localities and 8 susceptibles were studied.

Morphological studies show that *Erwinia amylovora* varies in size of the individual cells, and in size and form of the colony. Length of individual bacteria from strongly and moderately pathogenic isolates was found to vary from 0.93  $\mu$  to 1.40  $\mu$ , for weakly pathogenic ones from 1.50  $\mu$  to 1.71  $\mu$ . The length of bacterial cells appears to be a stable characteristic of the culture.

Marked variability of *Erwinia amylovora* in virulence was noted and correlated with morphological and some physiological characters.

Eosine-methylene-blue medium was found to differentiate between strongly and weakly pathogenic isolates in that the latter produce metallic lustre on slants of this medium.

Isolates were found to tolerate pH as low as 4.0 and as high as 8.8.

The upper thermal death point was determined to be between 45.1° and 48.3° C. for weaker isolates and between 48.3° and 49.5° C. for more pathogenic isolates.

The organisms survived on cover glasses for 24 to 36 hours with little apparent relation to humidity. In gauze strips the organisms lived from 4 to 10 days.

Cultural reactions of 10 isolates to sugars, alcohols, glucosides, amino acids, some proteins, fatty acids, and amides are reported. Variation was

<sup>6</sup> Vansell, G. H. Bee behavior as affecting pollination. (In press.)

noted among the isolates in their capacity to utilize certain of these chemicals.

Injections of asparagin into very resistant plants or into completely dormant plants seemed to promote infection upon subsequent inoculation.

Nine isolates grew in 50 per cent sucrose and one in 60 per cent sucrose. In glucose all isolates but one tolerated 14 per cent and one isolate grew in 28 per cent.

Dissociation of *Erwinia amylovora* was observed and studied. Aging of the culture invariably resulted in the appearance of rough forms. The rough form was found to be stable in ordinary solid and liquid media. The rough form was found to revert to the smooth type when passed from 4 to 6 times through 2 per cent sucrose or 1 per cent glucose nutrient broth.

The S type of *Erwinia amylovora* is the more virulent. The R type is avirulent for some susceptible shrubs and only slightly virulent to green pear fruits and succulent tips of pear seedlings.

R, as well as intermediate types, was obtained from old infections in nature.

Other variants were observed, including types with rhizoid and translucent colonies. The translucent type grows slowly in nutrient broth, requires frequent transplants in order to be perpetuated, and is avirulent to pear, apple, and certain shrubs.

Ten per cent sucrose broth decreases the invasive capacity of the culture but not by increasing the rough forms, while 2 per cent sucrose restores both attenuated and rough forms to the virulent state.

The influence of varying sugar concentrations, such as occur in the nectar of fruit-tree blossoms, and the dissociation of the organism, are believed to have an important bearing upon the rise and decline of epiphytotics of fire blight.

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# VARIABILITY OF POLYPORUS SCHWEINITZII IN CULTURE<sup>1</sup>

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## INTRODUCTION

Slight cultural differences within various species of wood-attacking fungi have been briefly mentioned by Long and Harsch (8) and by Fritz (3), but these workers, primarily interested in the diagnosis of decay, attached little importance to the variability they encountered. Schmitz (14) has described definite morphological and physiological differences between 4 isolations of *Fomes pinicola* (Sw.) Cke. Mounce (11), also working with *F. pinicola*, has discussed differences between cultures from a number of sources and ascribes these differences to individual variation rather than host influence. Aside from these studies, intraspecific variability in the Polyporaceae seems to have received little or no detailed attention, although the possibility of its occurrence often has been suggested.

When *Polyporus schweinitzii* Fr. was found causing a destructive root rot in 20- to 25-year-old plantings of northern white pine, *Pinus strobus* L., on unfavorable sites near Springwater, New York,<sup>3</sup> the possible implication of a heretofore unreported parasitic strain of either native or foreign origin could not be ignored. Cultures of this fungus, therefore, were requested from several American and foreign sources, and a number of isolations were made from diseased white pines at Springwater. A few isolations also were made from white pine and larch in New England. As this material accumulated it became increasingly apparent that there was no morphological basis for subdividing the species into local, parasitic, or host-specialized strains. Some of the cultures resembled each other rather closely, and all of them had a few characters in common, but with the exception of those taken from within a few feet of each other no two were identical, nor did those from any given host or locality manifest any special similarity.

In the past, *Polyporus schweinitzii* has been known principally as a cause of heart rot in butts of over-mature trees; with the disappearance of

<sup>1</sup> Presented to the Faculty of the Graduate School of the University of Pennsylvania in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

<sup>2</sup> This study was begun during the tenure of a Morris Arboretum Fellowship in the Department of Botany of the University of Pennsylvania, and was completed at the Morris Arboretum with the assistance of a fellowship granted by the Charles Lathrop Pack Forest Education Board. The writer also wishes to acknowledge his indebtedness to Dr. H. H. York and Dr. Conway Zirkle, to Edith Adams Childs for laboratory assistance, and to the many workers who furnished cultures.

<sup>3</sup> York, H. H. A study of resinosis and root rot in forest plantings of *Pinus strobus* and *P. resinosa*. Unpublished manuscript.

virgin stands it was expected to become relatively unimportant. Its destructiveness at Springwater and in a Douglas fir plantation near Biltmore, North Carolina, (6) suggests, however, that it may constitute a serious obstacle to the use of coniferous species in artificial reforestation, at least on certain sites. An investigation of its variability has, therefore, been undertaken to determine to what extent specialized strains may be responsible for damage to young trees. Certain preliminary observations are presented here. Final conclusions will be possible only when the results of field studies, already inaugurated, become available.

#### SOURCES AND DESCRIPTIONS OF CULTURES

It was the writer's practice to make several isolations from each sporophore or infected tree from which a culture of the fungus was desired. More than 100 isolations, representing 25 sporophores and 9 trees, were thus secured and cultured on nutrient agar under closely comparable conditions. With 2 exceptions, isolations from any given tree or sporophore were indistinguishable from one another, but differed more or less distinctly from those from any other source. It, therefore, was assumed that similar isolations from the same source were derived from the same individual mycelium, *i.e.*, were members of the same clone, and that dissimilar isolations were derived from genetically different mycelia. These mycelia, together with those obtained from other workers, will be referred to by the following numbers:

- 1 to 30, inclusive. From *Pinus strobus*; Springwater, N. Y.
31. From *Pinus strobus* (lesion on 0.5 cm. root of an 8-year-old planted tree); Honeoye Lake, N. Y.
32. " " " ; Weston, Ontario.
33. " " " ; Lake Timagami, Ontario.
34. " " " ; Harvard Forest, Petersham, Mass.
35. " " " ; Brattleboro, Vt.
36. " " *silvestris*; Stralsund, Germany.
37. " " " ; Skjaerningsfjell, Ringeby, Norway.
38. " " " ; Great Britain.
39. " " *rigida*; Medford, N. J.
40. " " *mughus*; Central Experimental Farm, Ottawa, Ontario.
41. " *Picea canadensis*; Lake Timagami, Ontario.
42. " " *sitchensis*; Moresby Island, British Columbia.
43. " " " ; Gahrenberg, Germany.
- 44 and 45. From *Pseudotsuga taxifolia*; Berlin, Germany.
46. From *Larix europea*; Island of Visingsö, Lake Vetter, Sweden.
47. " " *laricina*; Franconia, N. H.
48. " *Thuja plicata*; Vancouver, British Columbia.

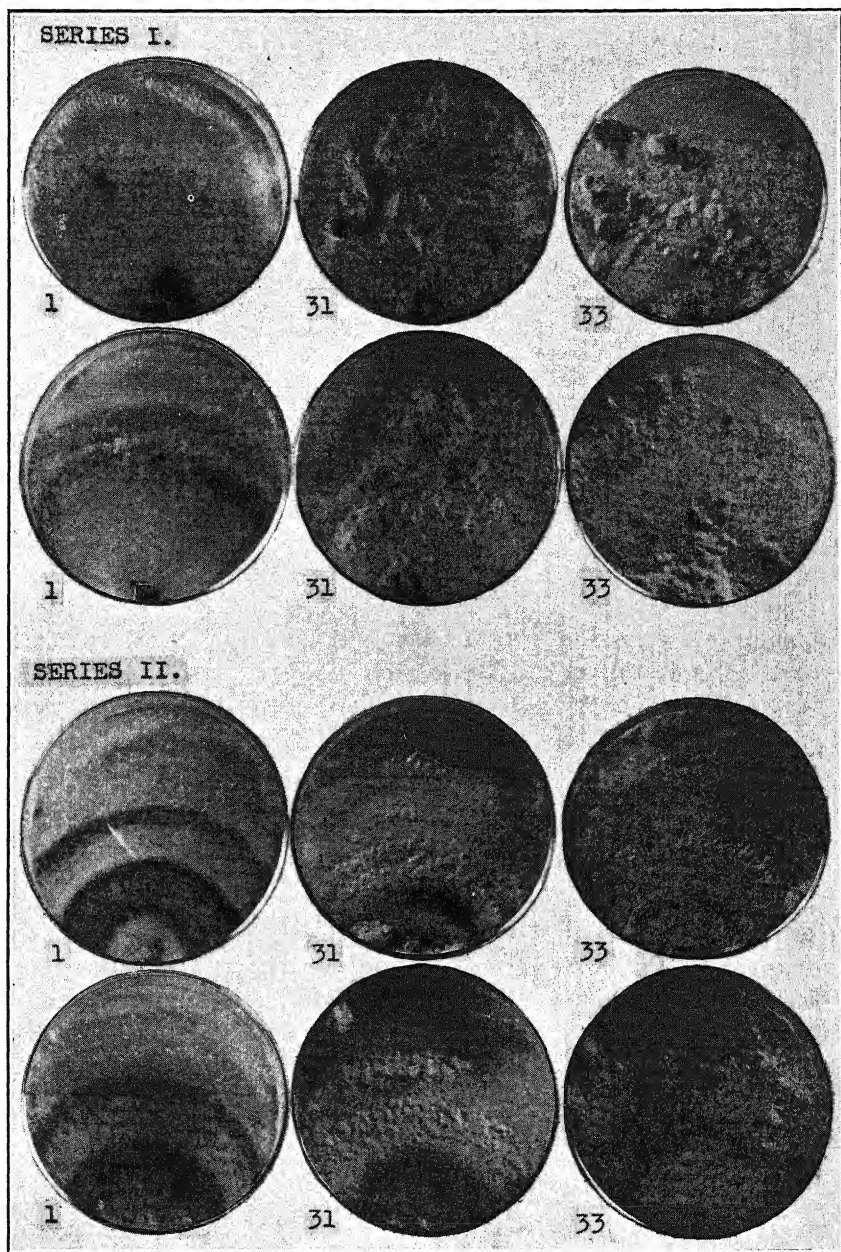


FIG. 1. Cultures showing differences between individual mycelia and between cultures of the same mycelium exposed to different environments, and similarities between parallel cultures of each mycelium. Numerals refer to the numbers given the various mycelia on pages 30 and 32.

49. Host not specified; Timagami Forest Reserve, Ontario.

50. Host not specified; Kyota, Japan.

One isolation of each mycelium was maintained as a stock culture from which all subsequent cultures of that mycelium, except when otherwise noted, were derived.

Cultures of these mycelia were under the writer's observation for periods of from 6 to 20 months, and in no instance was any alteration of cultural characters detected. At the same stage of development, cultures of any given mycelium differed in appearance only when they had been subjected to different environments. This is shown in figure 1, where growth characters of each of 3 representative mycelia are seen to be consistent in duplicate cultures raised under the same conditions (*i.e.*, in the same series) but are not consistent in cultures of the same mycelium in different series. The appearance of a given mycelium cultured in a given environment was determined entirely by the inherent nature of that particular individual mycelium and by the factors of that particular environment; *i.e.*, the cultural history of the material from which transfers were made had no appreciable effect upon the characters of the derived cultures.

Schmitz (14) and Mounce (11) have described and illustrated the line of demarcation that usually develops when different mycelia of *Fomes pinicola* approach each other on the same artificial substratum. An apparently identical reaction occurs between mycelia of *Polyporus schweinitzii*. When isolations from different sources were subcultured on the same slant or plate of nutrient agar, a well-defined dark line about 1 mm. wide usually appeared between them soon after the advancing margins had come in contact. This line did not form between subcultures taken from a single isolation or from similar isolations from any single source, nor did it form when monosporous mycelia were paired with other monosporous mycelia of either the same or different ancestry.

Where not otherwise specified, cultures were made on nutrient agar consisting of 20 g. of malt extract (either Fleischmann's Diamalt or Trommer's Diastasic) and 20 g. of agar per litre of distilled water. Sterilization was effected by autoclaving for 15 minutes at a pressure of 15 pounds. Growth was somewhat more luxuriant and fruiting was more frequent on media made with Diamalt. Trommer's extract was variable, some bottles being caramelized to a much greater extent than others, but the line of demarcation formed between mycelia from different sources was generally darker and more distinct when this brand of nutrient was used. In other respects these two brands of malt extract did not differ perceptibly in their effect on growth characters.

As Fritz (3) has remarked, *Polyporus schweinitzii* is quite sensitive to

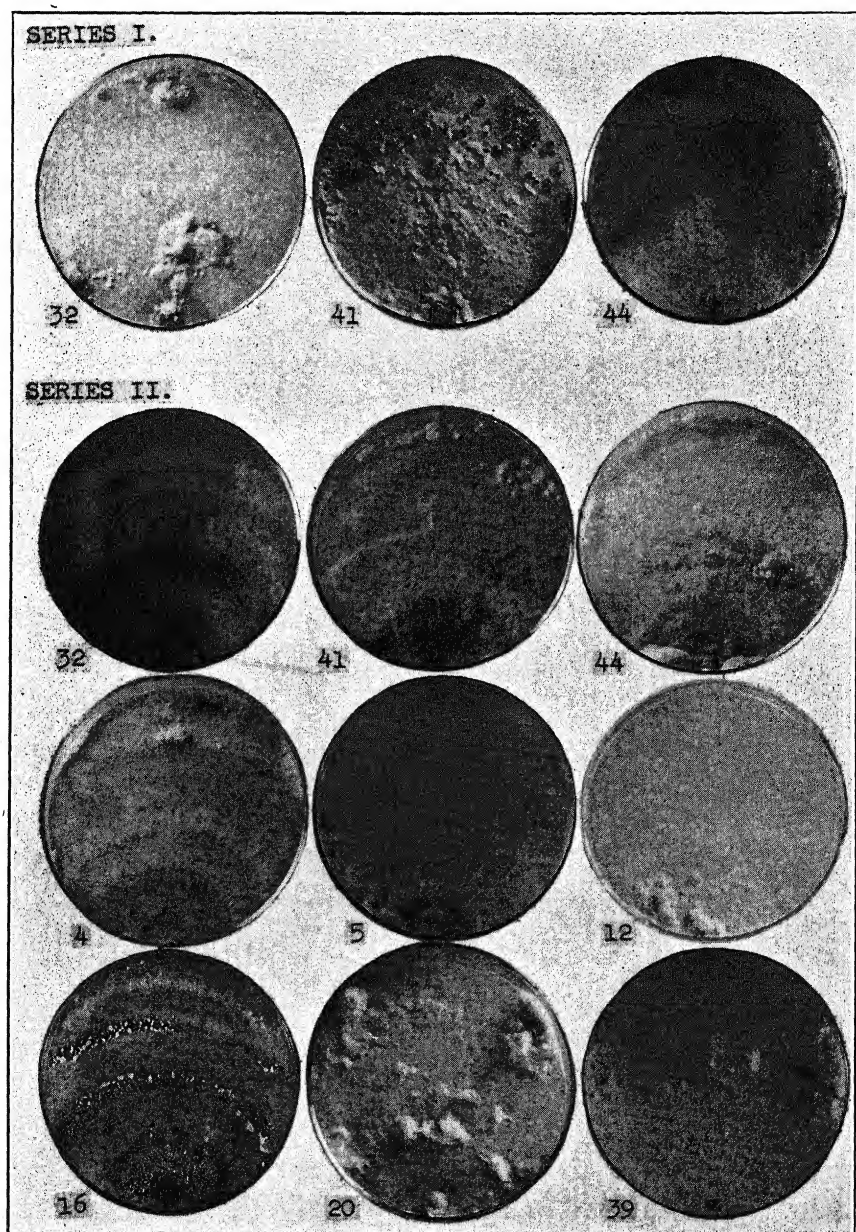


FIG. 2. Cultures showing differences between individual mycelia. Note sporophores in Nos. 16 and 41.

TABLE 1.—*Descriptions of some cultures of representative mycelia*

No.	Series	Color and character of growth			Concentric zonation
		Near inoculum	Intermediate region	Farthest from inoculum	
1.	I.	Sienna to olive brown; short pile (occasionally with 1 or 2 indistinct concentric zones of longer and darker pile).		Pale and sparse.	Indistinct. (Fig. 1).
	II.	Dark greenish-brown; uniform and moderately long pile.	Brown; uniform and moderately long pile.	Light red brown to lemon yellow; uniform and moderately long pile.	Quite distinct. (Fig. 1).
2.	I.	Yellow and light brown; fairly even mat.		Pale and sparse.	None.
	II.	Mostly greenish or reddish brown, with some lemon yellow to creamy yellow; regular and moderately long pile (occasionally slightly massed, tufted, or fluffy).			Fairly distinct.
3.	I.	Red brown; numerous very small tufts (some areas with short and fairly regular pile).		Lemon yellow.	Indistinct.
	II.	Light orange brown; fairly regular pile becoming very slightly and uniformly tufted.	Pale to lemon yellow; sparse to moderately long and irregular pile becoming slightly fluffy near edge of culture.		Fairly distinct.
4.	I.	Yellow brown (sometimes with one or two concentric zones of lighter yellow); short, matted pile.		Pale to lemon yellow.	Fairly distinct.
	II.	Olive to greenish or reddish brown; moderately long and uniform pile.			Fairly distinct. (Fig. 2).
5.	I.	Pale lemon yellow to reddish brown; an uneven mat.			Vague.
	II.	Yellow to orange; moderately long and irregular pile (some concentric zones of tufted, compacted, or fluffy growth.			Generally indistinct. (Fig. 2).
7.	II.	Mostly rather pale but with 2 or 3 fairly distinct concentric zones of lemon yellow to light brown; sparse except in regions of darker color, where regular pile is fairly well developed.			Fairly distinct.
12.	II.	Lemon yellow; short and irregular pile with some semicompact or slightly fluffy growth.	Pale and sparse.	Very pale; very sparse.	None. (Fig. 2).
18.	II.	Zones of reddish brown, short, and regular pile alternate concentrically with zones of dark yellow to orange, longer (sometimes slightly fluffy) pile.		Lemon yellow; moderately long and fairly regular pile (sometimes slightly fluffed).	Quite distinct.



TABLE 1.—(Continued)

No.	Series	Color and character of growth			Concentric zonation
		Near inoculum	Intermediate region	Farthest from inoculum	
31.	I.	Dark, mottled, yellow brown; irregular, matted pile with frequent fluffy growth.		Pale and sparse.	None. (Fig. 1).
	II.	Greenish to reddish brown; rather long and irregular pile.	Dark creamy yellow; small to medium large rounded tufts.	Pale to yellow green (with some reddish brown); sparse, irregular, or fluffy.	Fairly distinct. (Fig. 1).
42.	I.	Pale and sparse.	Pale and sparse.	Generally pale and sparse but with numerous long, cobwebby, lemon yellow to brown wefts or fluffy masses.	None.
	II.	Pale and sparse.	Pale and sparse.	Generally pale and sparse but with zones of loose, creamy wefts and some creamy to yellow green fluffs at edge of culture.	Rather vague.
44.	I.	Pale to yellow to reddish brown; very thin mat with short, sparse, pile (some lemon yellow, fluffy growth at edge of culture).			None. (Fig. 2).
	II.	Dark cream to orange; irregular (sometimes fluffy) pile.	Light to dark yellow green; pile irregular (fluffy in some central areas and near edge of culture).		Indistinct. (Fig. 2).
47.	I.	Yellow brown to reddish brown; short, irregular, pile and occasional small yellow fluffs.	Generally pale and sparse but usually with some pale lemon yellow growth near the edge of the culture.		Vague.
49.	II.	Greenish yellow, orange, and reddish brown; fairly long and regular pile (with a few small tufts).	A narrow zone of creamy, moderately fluffy growth.	Creamy to yellow green; sparse pile with some fairly well developed fluffy growth.	Rather indistinct.

light, growing more slowly and displaying darker and more varied coloration when exposed, even for very short periods, to indirect daylight than when kept in darkness. High humidity stimulates the development of fluffy superficial growth. Temperature, concentration and kind of nutrient, etc., also affect the appearance of cultures. The continuous nature of these environmental variables and the apparently great number of hereditary factors involved make it impracticable to describe in detail all the mycelia studied. Partial descriptions of a few representative mycelia cultured in Petri dishes are presented in table 1, however, to indicate the range and kind of cultural variations encountered during the study. In this table, and in figures 1 and 2, series I consists of cultures stored at a constant temperature of 22°<sup>4</sup> and exposed to electric light, but never to daylight, for a few seconds every 12 hours, while series II consists of cultures stored at room temperature (10° to 25°) and exposed to diffuse daylight for a few seconds every 4 or 5 days. Descriptions of series I were taken on the 19th day and photographs on the 40th day of growth. Descriptions and photographs of series II were taken on the 29th day of growth. From 2 to 20 cultures were made of each mycelium in each series. Cultural characters of each mycelium in each series were consistent in all cases where not otherwise indicated.

From the descriptions in table 1, and from the photographs in figures 1 and 2, it is apparent that differences between mycelia may sometimes be large enough to make difficult the diagnosis of wood decay by cultural methods. Identification may be facilitated by culturing on agar slants in ordinary test tubes and by storing cultures where they will be exposed to daylight of low intensity, since these conditions promote the formation of the more or less leathery mat and hasten the appearance of the "range of yellow tints" (3) typical of this species, but, even under these conditions, occasional mycelia are not readily identifiable on the basis of their cultural characters. The writer has not investigated the microscopic features of the various mycelia to a sufficient extent to determine their reliability as criteria of specific identity. Hasty and superficial observations indicate, however, that differences in microscopic characters are neither so great nor so frequent as are differences in gross cultural characters.

A number of mycelia were noticeably destructive to the agar, in some spots lowering the level of the surface 3 mm. or more below that of the rest of the culture (Fig. 3, 40K). Such lowering usually was confined to the region near the inoculum, but sometimes occurred in other portions of the culture. This destructive effect upon the agar, and the particular region in which destruction was visible, were quite as characteristic of certain mycelia as were the color and character of growth in culture. In cultures

<sup>4</sup> All temperatures recorded in this paper are in °C.



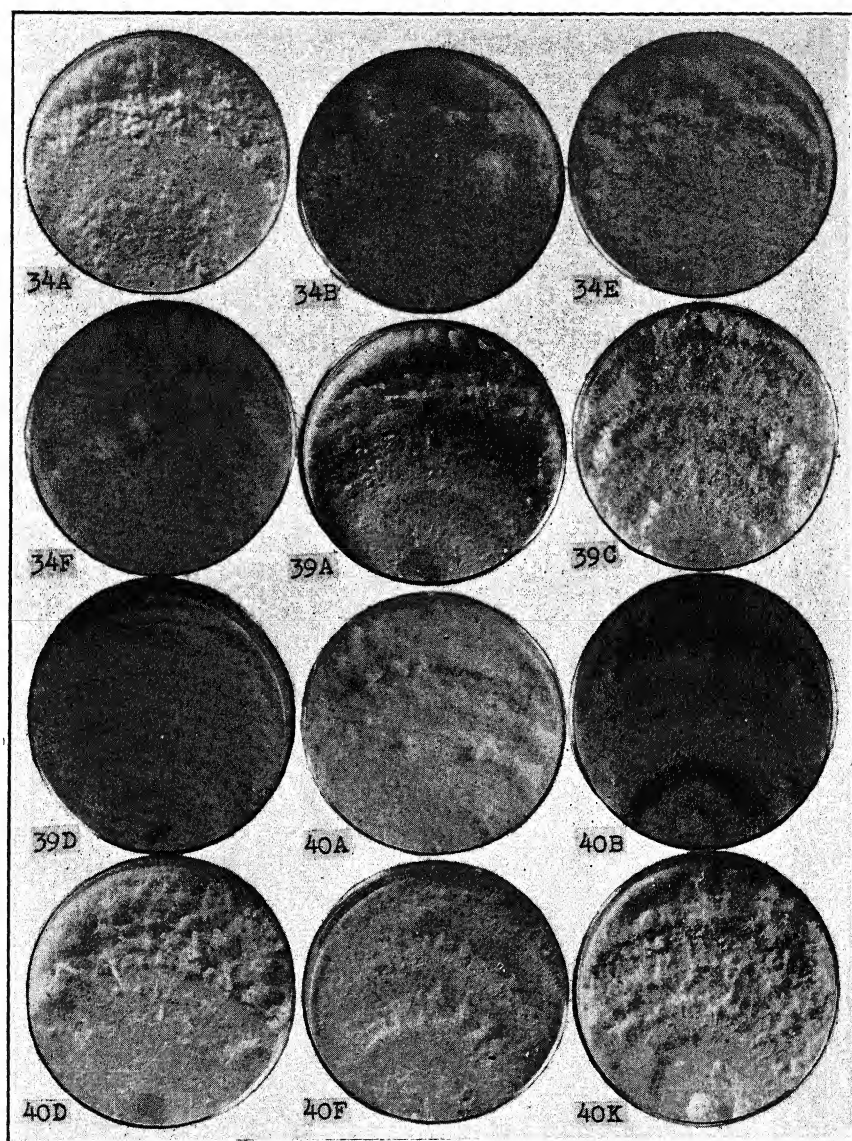


FIG. 3. Cultures showing individual differences between monosporous mycelia. Note sporophores in No. 40B and destruction of agar near inoculum in No. 40K.

of mycelia that did not alter the level of the surface of the substratum the agar retained its original gelatinous consistency until dry, but mycelia that caused any perceptible lowering of the agar surface also rendered the rest of the agar definitely crumbly, even in regions not adjacent to spots of visible destruction. Schmitz (14) has shown that some mycelia of *Fomes pinicola* secrete larger quantities of certain enzymes than are produced by other mycelia of the same species. The phenomenon just described suggests that mycelia of *Polyporus schweinitzii* also differ in enzyme production.

In only one instance during the entire study did repeated isolations from the same source fail to yield identical cultures. Two isolations each of Nos. 23 and 24 were made from a single sporophore. These mycelia differed so markedly in appearance as to be distinguishable from each other during very early stages of development. No. 23 was in no way noteworthy, displaying the usual cultural characters of the species and differing from most of the other mycelia only in relatively minor details. No. 24, however, became established very rapidly at ordinary temperatures and quickly filled the space above the agar slants with a permanently fluffy mass of hyphae. Portions of this mass ranged from a faint lemon yellow to a very light red brown, but most of it remained white throughout the life of the culture. In many respects this individual resembled some of the single-spore cultures described in a subsequent section.

No. 47, the only mycelium found in more than one individual host, was isolated several times from each of 3 wind-thrown larches separated from each other by distances of 20 to 30 feet. Since previous experience had shown that even neighboring hosts of the same species usually were infected by different mycelia, isolations from each of these trees were kept under close observation for about 6 months. At no time during this period were any greater differences detected between them than were to be found between parallel cultures derived from any single mycelium, nor was a line of demarcation formed when they grew together on a common substratum. Fruiting bodies have not been produced by these isolations and the possibility that they represent different mycelia belonging to a definite and perhaps specialized strain, therefore, has not been tested. Their homogeneity suggests, however, that a single mycelium has spread vegetatively to trees near the one originally infected.

#### RATE OF GROWTH ON NUTRIENT AGAR

During the growth of the cultures in series I, described in the preceding section, the radius of each culture was determined every 12 hours by measuring along the diameter of the Petri dish from the edge of the inoculum to the margin of the colony. Table 2 contains growth data derived from these measurements for 15 representative mycelia. (Nos. 4a and 35a were started

TABLE 2.—*Growth of representative mycelia in series I*

Mycelium No.	Days since establishment of cultures								Diff. between max. and min. on 8th day
	1	2	3	4	5	6	7	8	
	<i>Average radius of mycelium—mm.</i>								<i>mm.</i>
1. ....	11	17	23	28	35	43	51	58	7
2. ....	10	16	21	27	33	39	47	55	2
3. ....	10	16	22	29	37	45	53	61	8
4. ....	10	16	21	28	34	41	48	53	13
4a. ....	11	17	23	29	34	40	46	54	9
5. ....	10	16	23	30	37	44	52	60	3
31. ....	10	16	22	28	36	44	52	60	3
35. ....	10	15	21	27	33	40	47	55	3
35a. ....	10	15	21	27	34	40	48	56	3
38. ....	9	18	28	37	46	55	64	74	3
40. ....	8	11	14	18	22	25	30	34	13
41. ....	11	18	25	33	42	51	60	69	4
42. ....	12	21	30	38	46	55	64	74	6
43. ....	12	19	27	33	40	50	59	68	2
46. ....	13	22	30	38	46	54	62	70	2
48. ....	10	15	21	28	34	42	49	56	4
50. ....	10	16	21	27	34	41	49	56	6

one week later than the rest of the series, but were otherwise identical in origin and treatment with Nos. 4 and 35, respectively). Averages for each mycelium are based on 4 cultures of that mycelium. The principal difficulty encountered in studies of growth rates in this species was the difference in time required by the various mycelia for establishment on new substrata. In most instances, this difference was correlated with the character of the growth in cultures from which transfers were made. Other factors being equal, establishment was rapid when the transferred material included fluffs of loose aerial hyphae; less so when the aerial growth was compacted or matted; and slowest when the projecting hyphae were sparse and short. Mycelia that were to be transferred for comparative purposes were invariably cultured under the same conditions, but, because of inherent morphological differences, it was impossible to secure the same type of material for transfer from different mycelia. For this reason, the age of each mycelium in table 2 is computed from the time when the 4 cultures of that mycelium attained an average radius of 5 mm. (arbitrarily taken to indicate establishment) instead of from the time the transfers were made. Averages have been calculated, where necessary, by straight-line interpolation between the original semi-daily averages. The close correspondence between Nos. 4 and 4a, and between Nos. 35 and 35a, shows that, within rather wide limits, the

age of the agar is not an important factor, and that the arrangement of the data in this table may therefore be considered legitimate.

Differences between mycelia are clearly apparent in table 2. It is evident that Nos. 38, 41, 42, 43, and 46 have attained a greater radius and are growing more rapidly than the other mycelia, and that the growth rate of No. 40 is relatively low. Relative growth rates of mycelia cultured at higher or lower temperatures than series I were, however, frequently not consistent with the classification suggested by this table. For example, at 27° No. 43 grew more rapidly than No. 42, while at room temperature (Series II) No. 42 grew more rapidly than did No. 43. The cultures of No. 48 included in series II increased their average radius from 8 to 84 mm. in 14 days, while the cultures of No. 50 in the same series increased from 7 to only 65 mm. during the same period: at 27° the growth of No. 50 was appreciably more rapid than was that of No. 48. In every case where 2 mycelia differed significantly in growth rate at the extremes of the temperature range (app. 10° to 27°) to which they were exposed, but not at one or more of the intermediate temperatures, the mycelium that grew relatively rapidly at one extreme grew relatively slowly at the other. These observations indicate a difference between mycelia either in the form of the temperature-growth curve, or in the location of the maximum ordinate of this curve (*i.e.*, the optimum), or both.

Inclusion of the 3 mycelia from spruce in the group of rapidly growing individuals in table 2 is probably the result of coincidence rather than of any host-correlated relationship, since these 3 mycelia differed rather widely from one another in growth rate at other temperatures. Mycelia from Springwater, with one or two exceptions, maintained their relative rates of growth more consistently at the different temperatures to which they were subjected, and resembled each other more closely in absolute growth rates than did mycelia from other sources. This tendency toward similarity, even if not fortuitous, does not necessarily imply the existence of a specialized strain at Springwater. It may indicate only the closer degree of relationship that might logically be expected between isolations from a limited area as compared with isolations from widely separated points.

#### EFFECT OF ACIDITY ON GROWTH

Nutrient solutions adjusted to 9 different acidities were prepared by mixing 3 parts of 3 per cent Trommer's malt solution with 2 parts of KH phthalate buffers compounded according to the formulas of Clark and Lubs (2). The buffered solutions were placed in 38×200-mm. test tubes (app. 65 cc. of solution per tube) and autoclaved 15 min. at 15 lbs. pressure. The acidity of a sample from each lot was then determined potentiometrically, and the remaining tubes were inoculated with various representative my-

celia. One culture at each pH was made of each mycelium, except No. 1, which was cultured in duplicate. Twenty-three days after inoculation the depth of the mycelium and the acidity of the medium in each tube were determined (Table 3). Since in no case did the change in pH during the

TABLE 3.—*Effect of acidity on growth of mycelium of Polyporus schweinitzii*

Initial pH of buffered nutrient	Mycelium No.									Final pH
	1	1	8	31	40	42	47	49	50	
	<i>Depth of mycelium—mm.</i>									
2.5	9	10	5	5	8	8	6	6	15	2.6
3.0	18	18	13	12	15	15	10	10	28	3.0 to 3.2
3.5	26	27	28	30	22	34	16	25	45	3.6 to 3.7
4.0	40	43	41	44	45	46	37	39	52	4.2
4.3	42	44	50	45	46	52	50	42	52	4.5
4.7	44	46	55	39	40	50	43	42	55	4.8 to 4.9
5.1	43	43	53	36	7	48	39	46	55	5.2
5.4	22	21	28	17	9	20	20	34	33	5.4 to 5.6
5.7	10	10	10	7	4	11	7	12	15	5.6 to 5.9

growth of the fungus exceed 0.2, and since the 2 cultures of No. 1 at each acidity were reasonably consistent, the data in this table are considered approximately indicative of the effect of acidity on the growth of these mycelia. It will be seen that in every instance the optimum pH for growth was greater than 4.0 and less than 5.4, and in most cases probably lay between 4.2 and 5.0. Within these limits, however, the acidity permitting maximum growth was somewhat different for different mycelia. For example, Nos. 31 and 40 obviously made their best growth in more acid media than did Nos. 49 and 50.

#### RATE OF DECAY OF WOOD BLOCKS IN VITRO

Thirty-two white pine blocks (all from the same tree) approximately  $\frac{7}{8}$ "  $\times$   $\frac{3}{4}$ "  $\times$  4" were air-dried in the laboratory for several weeks, given individual numbers, weighed to the nearest half-gram, placed in 38  $\times$  200-mm. test tubes half full of water, and autoclaved 30 min. at a pressure of 15 lbs. They were then transferred to similar tubes, each of which contained a pure culture of the fungus growing actively in about 25 cc. of 2 per cent malt-extract solution, and stored in darkness at 22°. Two months later an additional 30 cc. of sterile distilled water was added to each tube. After a total incubation period of nearly 9 months the blocks were removed from the tubes, air-dried for 3 weeks, and reweighed. Average losses in weight, expressed as percentages of the original air-dry weights, were as follows:

Heartwood			
9 blocks inoculated with No. 1			$4.9 \pm 0.7\%$
7 " " " " 39			$3.7 \pm 0.7\%$
	Difference		$1.2 \pm 1.0\%$
Sapwood			
9 blocks inoculated with No. 1			$17.7 \pm 1.0\%$
7 " " " " 39			$24.9 \pm 2.7\%$
	Difference		$7.2 \pm 2.4\%$

The difference between the 2 sets of heartwood samples cannot be considered significant, since it is only slightly greater than its probable error.<sup>5</sup> The difference between the 2 sets of sapwood samples, however, is 3 times its probable error, and in spite of the small basis it may, therefore, be concluded that there probably is a real difference in the ability of these 2 mycelia to attack sapwood under the conditions of this experiment. Schmitz (14) presents evidence of similar differences between mycelia of *Fomes pinicola*. In his studies, as in the present instance, the relative order of destructiveness in heartwood is not the same as that in sapwood.

#### SPOROPOHORES IN ARTIFICIAL CULTURE

Eleven mycelia, including isolations from both sporophore tissue and decayed wood, fruited *in vitro* during the course of these studies. Nine of the 11 fruited under conditions to which most or all of the 39 sterile mycelia also were exposed, while the remaining 2 produced sporophores only under conditions to which less than half of the other mycelia were subjected. A few of the fertile mycelia fruited commonly: in others, fruiting was rare or sporadic.

In the series of wood-block cultures described in the preceding section, No. 39 formed sporophores consistently on sapwood but not on heartwood. It also fruited several times on white-pine sawdust and at the surface of cultures growing in a 2 per cent solution of malt extract. The only other mycelium cultured on these media was No. 1, which failed to fruit on any of them.

On Trommer's malt agar, Nos. 39 and 50 produced one sporophore each. Nos. 23, 30, 34, and 41 occasionally formed a few sporophores, and No. 40 fruited rather commonly. On this medium, sporophores usually appeared only in rather old cultures. Fruiting, therefore, might have been less sporadic had it been possible to retain the cultures longer.

Diamalt agar was apparently a somewhat better medium for sporophore

$$^5 \text{ Standard deviation} = \sqrt{\frac{\sum(f\hat{d}^2)}{n-1}}$$

$$\text{Probable error of average} = \frac{0.6745 \text{ S.D.}}{\sqrt{n}}$$

$$\text{Probable error of difference} = \sqrt{P.E._a^2 + P.E._b^2}$$

production. No. 1 fruited rarely; Nos. 9, 13, 23, and 41 fruited occasionally; and No. 16 fruited fairly regularly on this medium. No. 34 never produced sporophores in test-tube cultures but did so invariably in Petri dishes except when the layer of agar was quite thin. The most consistent and prolific fruiting encountered during the entire study was afforded by No. 40. This mycelium commonly produced sporophores in test-tube cultures: in Petri dishes, sporophore rudiments appeared soon after the surface of the agar was overgrown and functioning sporophores were often present in cultures only 3 weeks old.

The largest sporophores were those of Nos. 39 and 50, which averaged 1 cm. in diameter and ranged from 5 to 15 mm. in thickness: the smallest were those of No. 40, which averaged 3 mm. in diameter and 2 mm. in thickness. Color varied from yellow (Nos. 34 and 40) to reddish brown (No. 50) or dark brown (No. 39). Neither stipes nor recognizable pilei were present in any instance. Sporophores that developed above the culture medium consisted principally of short spines (produced by the majority of the fertile mycelia), small and disarticulated lamellae (Nos. 34 and 40), or longer hydroid processes, frequently once- or twice-dichotomized (formed only by No. 39). Pores occurred infrequently, and then only in sporophores borne laterally, as on wood blocks and vertical agar slants, or vertically, as in inverted Petri dishes. Average pore diameter varied from less than 0.5 mm. in No. 40 to slightly more than 1 mm. in No. 39. Small pores were approximately circular but the larger ones were quite angular. Young fruiting bodies that had recently become spiny or lamellate could sometimes be induced to form pores by reversing their orientation with respect to gravity. Neither sporophore production nor pore formation appeared to depend on exposure to daylight. The microscopic structure of hymenia in artificial culture did not differ essentially from that of hymenia developed under natural conditions. Sporophores formed by different mycelia displayed some microscopic dissimilarities—for example, cystidia of No. 34 were relatively large and sometimes terminally swollen, while those of No. 40 were smaller and of more nearly uniform diameter throughout. These variations, however, were no greater than those occurring normally in "wild" sporophores. It seems probable that the well-known variability of the sporophores of many species of Polyporaceae under natural conditions (9, 15) is simply another example of differences between individuals.

#### MONOSPOROUS AND POLYSPOROUS MYCELIA

Basidiospores of *Polyporus schweinitzii* are so small and fragile that it is extremely difficult to secure uninjured single spores by Hanna's dry needle method (4), even with micromanipulation apparatus. Progressive dilution of material from spore prints is much less laborious, but the serviceability



of this method is impaired by the persistence with which many of the spores remain aggregated in small groups throughout the process of dilution. The procedure devised by Mounce (11),<sup>6</sup> however, proved quite satisfactory and in a slightly modified form was followed in obtaining all single-spore cultures except those from No. 39, which were secured by dilution. Polysporous cultures were obtained by scraping an uncontaminated spore print with a sterile spear-pointed needle and planting the material thus obtained on a slant of nutrient agar. Both monosporous and polysporous cultures were derived in all cases from spores produced by pure cultures of the fungus.

Fresh spores germinated readily, and numerous monosporous cultures were obtained from Nos. 34, 39, 40, and 50. These monosporous cultures resembled the stock mycelia in many respects. The mat developed on the surface of the agar seemed, however, generally thinner and more delicate, and superficial growth was often much fluffier. The following, cultured under the same conditions as series II, are fairly representative (Figure 3):

No. 34A.<sup>7</sup> Orange brown and rather irregular pile with numerous short, fluffy, pale lemon yellow tufts (Fig. 3).

No. 34B. Growth sparse and pale near inoculum; becoming pale yellow and loosely fluffed at other side of culture (Fig. 3).

No. 34D. Orange to dark olive brown pile near inoculum; pale lemon and loosely tangled fluff at other side of culture.

No. 39A. Reddish brown near inoculum; reddish brown pile with numerous short tufts and fluffy cream-color masses at other side of culture (Fig. 3).

No. 39B. Reddish brown near inoculum; a mixture of light orange and dark cream at far side of culture.

No. 39C. Zones of pale, sparse growth alternate with zones of orange pile near inoculum and with zones of cream-color fluffy growth at far side of culture (Fig. 3).

No. 40A. Growth near inoculum mostly pale and scantily fluffy; rest of culture consists of light to dark yellow fluff (Fig. 3).

No. 40B. Sparse and pale near inoculum, with some destruction of agar; rest of culture dark lemon yellow to olive or red brown (Fig. 3).

<sup>6</sup> "A sporophore was removed from a culture with sterile forceps and quickly fastened to the cover of a sterile Petri dish by means of a drop of melted agar. Then the cover was removed from a Petri dish containing lactose gelatine, the cover bearing the sporophore was substituted, and slowly rotated. Then the cover was replaced and as many Petri dishes as desired were inoculated in this way. The plates were kept at room temperature for from four to five days. By that time, the germinating spores appeared as tiny depressions in the surface of the clear gelatine. A circle was drawn around each mycelium, it was examined under the microscope, and if it proved to be monosporous, and if there were no other spores within the circle, it, surrounded by a small square of gelatine, was removed with a sterile spear-shaped needle, placed on a potato-dextrose or malt agar slant, and grown at room temperature."

<sup>7</sup> Numeral refers to parent mycelium—*e.g.*, No. 34A is the first monosporous mycelium secured from No. 34.



No. 40C. Mostly sparse and pale near inoculum; rest of culture raggedly fluffy and pale yellow to dark creamy yellow or light orange.

No. 40F. Zones of sparse and pale growth alternate with zones of short, lemon yellow tufts (Fig. 3).

No. 40K. Most of agar destroyed in immediate vicinity of inoculum; rest of culture consists of light to dark yellow fluff (Fig. 3).

From these descriptions, and from table 4 and figure 3, it will be seen

TABLE 4.—*Growth of representative mycelia of both monosporous and natural origin (Series II)*

Mycelium No. <sup>a</sup>	Age of cultures—days				Diff. between max. and min. on 16th day
	7	11	16	21	
	<i>Average radius—mm.</i>				<i>mm.</i>
1. ....	15	27	49	77	2
3. ....	2	13	41	69	4
4. ....	18	33	64	82	4
34. ....	6	17	41	63	3
34A. ....	22	39	65	b	0
34C. ....	18	36	67	b	8
34D. ....	22	40	68	b	3
34F. ....	16	28	48	63	4
39. ....	8	25	56	80	0
39A. ....	9	27	55	79	2
39C. ....	12	26	54	74	2
39D. ....	10	23	38	54	8
40A. ....	12	32	63	83	2
40B. ....	13	31	60	81	7
40E. ....	12	32	69	b	2
40F. ....	13	30	56	76	7
40K. ....	11	29	62	b	1
41. ....	13	32	63	82	7
43. ....	6	19	39	62	9
46. ....	7	23	56	90	2
48. ....	8	24	51	84	6

<sup>a</sup> Data for each mycelium are based on 2 cultures.

<sup>b</sup> Entire surface of culture overgrown (radius greater than 90 mm.).

that differences between monosporous mycelia are of the same general type and of about the same order of magnitude as those between isolations secured from different sources in the field. Cultural differences are ill-adapted to quantitative expression, and an exact comparison of the extent of variability in the different groups of monosporous mycelia of common descent is, therefore, impracticable; in general, however, it may be said that mycelia having the same immediate ancestry appeared to differ less than did those of more diverse descent. Individual differences were apparent, however, even be-

tween mycelia descended from the same sporophore. When cultured under the same conditions, hyphal-tip transfers from opposite sides of a given monosporous colony were culturally identical.

Twenty-four of the 41 monosporous mycelia secured from No. 40 produced sporophores. From one of these fertile monosporous mycelia (No. 40L) 33 single-spore cultures were made, of which 30 produced sporophores. These third-generation cultures resembled each other very closely, much more so than did those of the preceding generation, but could not be considered identical in appearance. The only other sporophores that appeared in either monosporous or polysporous cultures were developed by a few descendants of Nos. 39 and 50. Some of these were presumptively of single-spore origin, but a few of the supposedly monosporous cultures (including the fertile ones) derived from No. 39 showed striking similarity to polysporous cultures from the same mycelium: In view of the defects of the dilution method (used with spores from this mycelium only) the monosporous origin of these cultures is doubtful.

Polysporous cultures from Nos. 34 and 40 were similar in general appearance to isolations secured in the field, but polysporous cultures from Nos. 39 and 50 displayed none of the gross characteristics of *Polyporus schweinitzii*. The hyphae in the latter two were hyaline, giving cultures on malt agar a dirty grey appearance, and were almost entirely confined to the medium within 1 mm. of the surface of the agar slants. Polysporous cultures from No. 39 produced a few short aerial hyphae that looked like very fine cotton lint sparsely sprinkled on the surface of the agar. Those from No. 50 produced no aerial growth. In other respects these cultures were indistinguishable from one another. After repeated transfers, several of the polysporous cultures from No. 39 developed thin, irregular, dark reddish brown mats on the surface of the agar slants: such cultures invariably produced sporophores.

#### DISCUSSION

Morphological or cultural characters and more purely physiological qualities are not necessarily correlated (1). Nevertheless, the wide range of variability apparent between the cultures from Springwater, a range almost as great as that found between cultures from widely separated points and unlike hosts throughout the Northern Hemisphere, indicates that damage in this locality cannot now be attributed definitely to infection by a race or strain of special virulence. The planting stock used in the infected areas at Springwater came from nurseries in which seedlings from European nurseries had been transplanted, and other plantings in the immediate neighborhood have been made with stock of European origin, but the large number of different mycelia isolated from the infected trees precludes the possibility of infection having originated entirely by vegetative spread of the organism

from one or a few foci in nursery beds. Fruiting bodies of *Polyporus schweinitzii* have been rare in these plantings until quite recently.<sup>8</sup> It seems unlikely, therefore, that a few original infections by a virulent strain have been spore-propagated, with consequent morphological diversification of the organism, to a sufficient extent to account for the large and varied population of this species now present in these areas. Wean<sup>9</sup> has shown that infection may occur in very young trees, but his studies also indicate rather definitely that parasitic activity of this fungus is favored by certain conditions, such as those at Springwater, which are suboptimum for white pine. Pending the completion of inoculation studies in the field it must accordingly be concluded that the *Polyporus schweinitzii* epiphytotic in this locality probably is attributable to edaphic factors that have permitted extensive infection by native representatives of the species.

The possibilities of future specialization created by the wide variability exhibited by this fungus must not be overlooked. It may be assumed that the majority of the individuals constituting this species are now adapted to a quasi-parasitic life in the basal heartwood of older trees, but extensive infection of younger trees growing under more or less unnatural conditions might conceivably result in the gradual selection of lines best adapted to such existence and a consequent increase in the average virulence of the species to planted trees even on good sites. The likelihood of such a change and the time necessary for it to occur must remain problematical, but the immediate values involved are great enough to justify the development and adoption of preventive and control measures.

Variability of the type described in this paper is by no means peculiar to the Polyporaceae. Recent pathological literature contains numerous references to similar instances of intraspecific variability in widely separated species of fungi. The work of Magie (10) on *Coccomyces hiemalis*, Johnson and Valleau (7) on *Thielaviopsis basicola*, Palmiter (12) on *Venturia inaequalis*, and Hansen and Smith (5) on *Botrytis cinerea* may be cited as random examples. All of these workers observed differences in cultural appearance between isolations, and in some cases also found differences in such characters as growth rate, spore production, size and shape of conidia, pathogenicity, reaction to toxic substances, and reaction to acidity of the medium. None of these differences were correlated with the host or locality from which the isolations were made. Palmiter's (12) statement seems generally applicable to all of the above-mentioned species: "No two of the 36 isolations studied appeared exactly alike when grown under standardized conditions, yet duplicate cultures could easily be recognized. . . . *V. inaequalis*

<sup>8</sup> York, H. H. A study of resinosis and root rot in forest plantings of *Pinus strobus* and *P. resinosa*. Unpublished manuscript.

<sup>9</sup> Wean, R. E. The parasitism of *Polyporus schweinitzii* on seedling *Pinus strobus*. Unpublished manuscript.

is not a homogeneous species or one composed of a few well-defined forms with definite cultural and pathogenic reactions but is one made up of many strains that differ in various degrees in their morphologic and physiologic characters." Research on such highly heterozygous species can be of maximum usefulness only when the sampling errors inseparable from studies of varied populations have been reduced by the use of a large and representative basis. Small bases may be sufficient in species characterized by slight variability, but in most instances a high degree of variability must be assumed until investigations of numerous isolates have shown the species to be essentially uniform for the character in question. It seems probable that discrepancies between quantitative data secured by competent workers often may be charged directly to the basing of studies on only one or a few individuals from species in which wide variation may exist. For example, Lagerberg and Melin,<sup>10</sup> Robak (13), and Weis and Nielson (16) disagree rather widely as to the optimum pH for growth of *Fomes annosus* (Fr.) Cke. in artificial culture. This disagreement may be the result of differences in experimental conditions, but the observations of Palmiter (12) and the present writer suggest that it is caused by differences between the individual mycelia used in the various studies.

The term "strain" often is employed by pathologists to designate a single original isolation and its subcultures (with the exception of saltants, etc.). Its use in this particular connection is unfortunate, since it frequently leads the reader to an erroneous conception of the relationships involved, even when it does not indicate a misunderstanding on the part of the user. "Strain" suggests a racial grouping<sup>11</sup> of genotypically distinct individuals that are homozygous for one or more physiologic or cultural characters serving to distinguish them from other members of the species. In many instances the term is, of course, correctly used, but it is not applicable to any one of the different mycelia of *Polyporus schweinitzii* herein described. Genetic irregularities and the frequent occurrence of asexual reproduction obscure the individualization of fungi, but in this group, as in any other group of organisms, individuals are the concrete units into which such abstract conceptions as strains and species are ultimately resolvable. The situation is made more complex but is not fundamentally altered by such anomalous phenomena as mixochimaeras (heterocaryotic cells), "discontinuous variations", and physical discontinuity of individual genotypes. For practical purposes, separated fragments of an organism or new colonies

<sup>10</sup> Not seen by the present writer; *vide* Weis and Nielson (16).

<sup>11</sup> "When the isolates are compared on various culture media and under different environmental conditions they fall into a number of groups each of which contains isolates which are alike in their cultural characters and cultural behaviour. Each of these groups constitutes a Strain or Race, and equates with Lotsy's 'Jordanon'. . . . Cultural races may be equated with biological races in the more strict sense." Brierley (1).

that have originated from accessory spores of the same genotype can be accorded no greater claim to separate individual ranking in Nature than *in vitro*.<sup>12</sup> Brierley's isolates, then, do not necessarily represent different individuals in all cases, although for the sake of convenience they must often be considered to do so. "Mycelium" has been used throughout the present paper in a sense that the writer considers equivalent to "clone". "Genotype" or, less accurately, "individual", might be similarly used. Choice of terms must frequently depend, as in the present instance, on convenience of expression and on the extent to which relationships within the species in question are known. Whatever the terminology, the essential fact remains that some species of fungi, of which *Fomes pinicola* (11) and *Polyporus schweinitzii* may be cited as examples among the Polyporaceae, are made up of individuals that differ from one another in many respects, and that investigations of such species must, therefore, include a sufficient number of individuals to permit an approximate determination of the specific average and range of variability.

#### SUMMARY

Cultural studies of 50 different mycelia of *Polyporus schweinitzii*, secured from various coniferous hosts and widely separated points throughout the Northern Hemisphere, show that this species is made up of individuals that differ rather widely from one another in appearance in culture, production of sporophores, growth rate on nutrient agar, reaction to acidity, and apparently also in ability to cause decay of wood. Individual differences also were found between monosporous mycelia secured from spores produced by pure cultures of the fungus. There was no evidence of the occurrence of local or host-specialized strains within this species, and it therefore seems probable that the serious damage which the fungus is causing to planted white pine near Springwater, N. Y., is attributable to site conditions rather than to any special virulence on the part of the fungus in these plantings. The differences shown to exist between individuals of this species are comparable to those demonstrated by Mounce (11) for *Fomes pinicola*, and indicate that research on such variable species can produce generally applicable results only when a large number of individuals are studied.

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<sup>12</sup> "Each separate pure culture made by direct isolation from fresh material, whether a number of cultures are made from a single lesion or from one or more host plants, I term an Isolate. . . . Each isolate is an individual line and sub-cultures are merely duplicates of that isolate or line. The isolate is the nearest equivalent to Lotsy's 'species.'" Brierley (1).

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# STUDIES CONCERNING THE REACTION OF BARLEY TO TWO UNDESCRIBED PHYSIOLOGIC RACES OF BARLEY MILDEW, *ERYSIPHE GRAMINIS HORDEI* MARCHAL<sup>1</sup>

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## INTRODUCTION

Salmon (26, 27) lists 59 species in 26 genera of grasses as hosts of *Erysiphe graminis* DC. Marchal (17) was the first to show that this species contained several "*formes spécialisées*", or physiologic varieties, differentiated by their host specialization. From further investigations by both Salmon (28, 29, 30, 31) and Reed (19, 20, 21, 23), it is evident that different varieties occur on each of the cereals, oats, barley, rye, and wheat. Moreover, distinct varieties occur on each of the following genera of grasses: *Agropyron*, *Bromus*, *Dactylis*, and *Poa*. In general, each variety is closely confined to species of a single genus. Two of the varieties, *Hordei* and *Tritici*, are known to contain physiologic races. Mains and Dietz (15) first demonstrated this for the *Hordei* variety, and Mains (14) has shown this to be true for the *Tritici* variety. The present investigation is limited to 2 hitherto undescribed physiologic races of the *Hordei* variety.

Genetical studies concerning the resistance of barley to powdery mildew are few. In 1907, Biffen (1) studied a cross between a resistant and a susceptible variety. Recent genetical investigations are those of Dietz (5), Honecker (8, 9), and Briggs (2), each of whom used different physiologic races of *Erysiphe graminis hordei* as inoculum. Briggs also studied mildew resistance in relation to other factors.

## MATERIALS AND METHODS OF STUDY

Seed of the barley species, varieties, and crosses was furnished by E. B. Mains. The study of crosses was started with the F<sub>2</sub>. The mildew was obtained from infected barley growing in the greenhouses at the Botanical Gardens of the University of Michigan.

In studies of the seedling reactions, approximately 10 to 15 seeds of each of the varieties to be tested were planted in 3½ inch pots on the greenhouse

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bench. When the first two or three leaves were showing the plants were sprayed with a fine mist of water and then were inoculated by shaking heavily mildewed plants over them. A uniform distribution of conidia was thus obtained on all leaves. The plants were then covered with wet muslin suspended on wires over the pots. When this was done in late afternoon a fine dew occurred on the leaves the next morning. At this time the plants were often reinoculated, thus insuring a more abundant infection. The wet cloth was removed at the end of about 24 hours from the time of first covering. In warm, sunny weather it covered the plants only at night because of the excessively high day temperature.

In the winter, when the temperature was more uniform in the greenhouse, 65° to 75° F. during the day and 55° to 65° at night, good infection was obtained merely by shaking heavily mildewed plants over the varieties to be inoculated without covering the plants with wet cloth. The inoculum was obtained from seedling plants of highly susceptible varieties, such as Oderbrucker C. I. 940.

Notes were taken about 10 to 20 days after inoculation, when the mildew had reached its fullest development. As the time approached for taking such notes the plants were no longer watered from above. In this way, washing of the spores from the leaves was considerably reduced and readings were facilitated.

Five types of reaction of the host varieties were recognized, similar to those distinguished by Mains and Dietz (15), Dietz (5), and Mains and Martini (16) in their studies of the powdery mildew of barley. These types are as follows:

0. Highly resistant. Macroscopically, no mildew evident; chlorotic or necrotic spots, or smaller areas appearing as white flecks may be developed by some varieties.

1. Very resistant. Slight to moderate development of mycelium evident macroscopically, but with little or no sporulation; chlorotic or necrotic spots, or brownish areas developed by some varieties.

2. Moderately resistant. Moderate to abundant development of mycelium accompanied by a slight production of conidia; chlorotic or necrotic spots formed by some varieties.

3. Moderately susceptible. Moderate to abundant development of mycelium accompanied by moderate sporulation.

4. Very susceptible. Abundant mycelium developed, accompanied by abundant sporulation.

The "X" type of reaction often encountered by workers with cereal rusts was seldom found in tests of barley reacting to pure physiologic races of the mildew. This is essentially a heterogeneous type of infection in which several, if not all, of the types of infection from 0 to 4 are found on the same



leaf. Plants encountered in crosses between susceptible and resistant varieties that gave this or a type 2-3 reaction are listed in the present paper as "intermediate" in reaction.

A mixture of several races usually is indicated by one or more of the differential varieties giving two or more types of reaction at the same time, much as one encounters in the "X" type of reaction. In such instances pure cultures of the races present may be obtained by resort to one of several methods. Mains (12) and Newton and Johnson (18) have given an excellent discussion of the separation of individual races from cultures containing several physiologic races in certain of the cereal rusts. Much of the technique that they describe is applicable also to powdery mildew of barley.

In the present study resort was made constantly to single pustules in order to obtain pure cultures of the races under consideration. Seedlings known to be susceptible to several races were placed on the greenhouse bench, where mildewed barley also was present. Such healthy seedling plants usually were placed 10 to 30 feet from the mildewed plants and exposed for a day to conidia that were in the air of the room. They were then removed to a compartment where there was no mildew. After the usual period of incubation signs of infection appeared on the leaves as white flecks. As the inoculation had been scattered, plants with a single white fleck of mildew could be easily isolated. If other leaves of the plant also were flecked, they were removed, leaving in every case a single white fleck or mildew pustule to a plant. These selected plants were placed in separate moist chambers or compartments and kept from further exposure to conidia. When a single pustule began to sporulate, transfers of the conidia were made with the moistened blade of a spatula to other healthy susceptible plants. The mildew was then multiplied.

#### REACTION OF BARLEY TO PHYSIOLOGIC RACES 6 AND 7 OF *ERYSIPHE GRAMINIS HORDEI*

The results presented in this and the following section were obtained during investigations conducted during the winter (November-March) and spring (April-May), 1933-1934, and the winter of 1934-1935 in greenhouse studies. In winter tests, a day temperature of 65° to 75° F., and a night temperature of 55° to 65° was maintained. In the spring the temperature occasionally went as high as 90° during the day.

Two physiologic races were isolated that did not agree with those previously described. These are designated as physiologic races 6 and 7.

#### Differential Host Varieties for the Identification of Seven Physiologic Races of Barley Mildew

Mains and Dietz (15) used the reactions of 4 barley varieties, Black Hull-less C. I. 666, Goldfoil C. I. 928, Nepal C. I. 595, and Peruvian C. I. 935, in

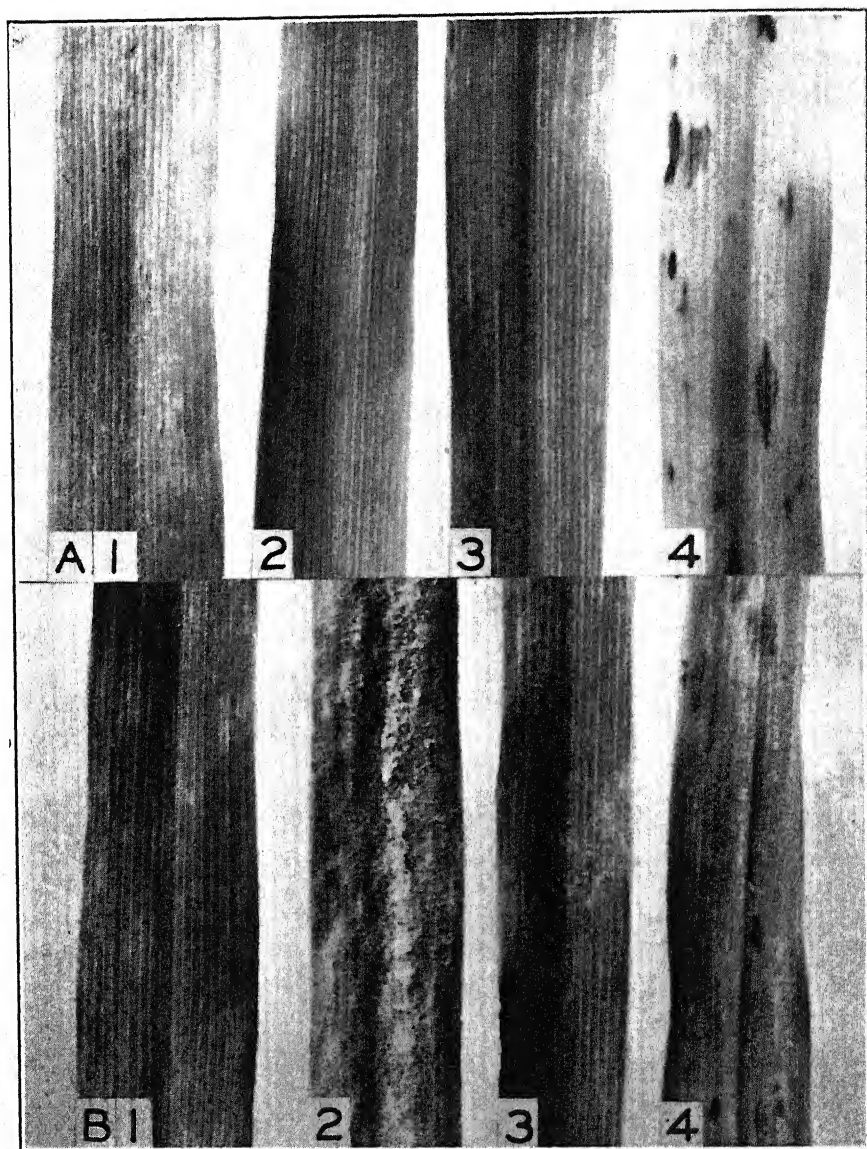


FIG. 1. Reaction of seedling leaves of four differential varieties of barley to two physiologic races of *Erysiphe graminis hordei*.  $\times 3$ . A. Four varieties inoculated with p. r. 6. (1) Nepal C. I. 595, type 1, very resistant, slight development of mycelium. (2) Goldfoil C. I. 928, type 0, highly resistant. (3) Peruvian C. I. 935, type 0, highly resistant. (4) Black Hull-less C. I. 666, type 0, highly resistant with conspicuous necrotic spots. B. Same four varieties inoculated with p. r. 7. (1) Type 1-, very resistant, very slight development of mildew. (2) Type 3+, very susceptible, abundant development of mycelium and moderate sporulation. (3) Type 1, very resistant, slight development of mycelium. (4) Type 1-, very slight development of mycelium, definite necrotic spots.

order to separate the 5 physiologic races of powdery mildew they isolated. It is necessary to add Heil's Hanna 3 C. I. 682 to this list of differentials in order to separate races 6 and 1. The reactions of these 5 varieties to the 7 physiologic races are presented in table 1. Figure 1 illustrates the reactions of 4 of the differential varieties to races 6 and 7. No decided changes in reaction to either race 6 or 7 were noted between winter and spring tests, under the conditions of this study. Since no tests were conducted during the summer, in either greenhouse or field, it is not known whether these reactions will be changed under such conditions.

TABLE 1.—*Reactions of five differential varieties of barley in the seedling stage to seven physiologic races of Erysiphe graminis hordei*<sup>a</sup>

Variety	C. I. <sup>b</sup> No.	Type of reaction to physiologic race						
		1	2	3	4	5	6	7
Black Hull-less .....	666	0-2	1-2	3+	4	3	0-2	0-1
Goldfoil .....	928	0	0	0	0	4	0	3-4
Heil's Hanna 3 ...	682	1-2	4	4	3-4	4	3-4	3-4
Nepal .....	595	1-2	4	4	4	4	0-1+	0-1+
Peruvian .....	935	0-1	1	3-4	1	4	0-1+	0-1+

<sup>a</sup> The data for the reactions of these varieties to races 1 to 5 inclusive were obtained from Mains and Dietz (15, p. 233).

<sup>b</sup> Accession number of the Division of Cereal Crops and Diseases, United States Department of Agriculture.

### Reactions of Seedling Plants

Both physiologic races 6 and 7 were tested on seedlings of a number of varieties several times during the winter and spring, under the differing environmental conditions then prevalent. In general, most of the varieties reacted to race 6 much as they did to race 7 (Table 2). However, two varieties, Goldfoil C. I. 928 (Fig. 1, A2 and B2) and Hanna C. I. 906 gave reactions that differed decidedly. Both varieties were extremely resistant to race 6, giving a 0 reaction. To race 7 they were very susceptible, giving reactions of 3 or 4.

A number of varieties were extremely resistant to both races in winter and spring tests, for the most part consistently giving 0 reactions. Such varieties were Albacaete C. I. 1128, Arlington C. I. 702, Chevron C. I. 1111, Chinerme C. I. 1079, Duplex C. I. 2433, Monte Cristo C. I. 1017, C. I. 2444, M756, M757, and Psaknon B81. All, except Psaknon B81, remained green and healthy with little or no flecking, no chlorosis, and no necrosis. Psaknon showed occasional flecking and a slight browning of the leaf tissue.

A larger number of varieties were classed as very resistant, yet varying in reaction from 0 to 1. With Chilean C. C. I. 1432, Chilean D. C. I.

1433, Coast C. I. 276, Peruvian C. I. 1131, C. I. 1080, C. I. 2416, and Orge Fourragère B102, there was a sparse development of mycelium occasionally with a slight brown discoloration of the leaf surface under the mycelium. Sometimes this was accompanied by a slight flecking, but never with any decided necrosis of host tissue. Other varieties, such as Kwan C. I. 1016, Sulu C. I. 1022, C. I. 1021, and No. 22 B69, also gave 0 to 1 reactions, but with a conspicuous necrosis of leaf tissue. Necrotic areas several millimeters in length often were produced. In the center of some of these a little tuft of mildew mycelium occasionally was observed. Black Hull-less C. I. 666 (Fig. 1, A4 and B4) and Minsturdi M784 also were resistant, with necrosis accompanying the slight development of mycelium.

Several other varieties, classed as very resistant, varied slightly, depending on whether the tests were conducted in winter or spring. Nepal C. I. 475 and Arequipa C. I. 1256, which gave reactions as high as 2 to physiologic race 6 during the winter, gave a type-1 reaction in the spring. Juliaca C. I. 1114 varied in the winter readings, sometimes giving a type-1 reaction to race 6 and at other times as high as 3. In the spring the reactions were never higher than 1. Peru C. I. 653 sometimes gave a type-2 reaction in the winter, while in the spring this variety was slightly more resistant. The reverse, that varieties resistant in the winter were less resistant in the spring, was seldom true.

Many varieties were noted as susceptible to both races during winter and spring. In the winter some of these were moderately or highly susceptible, giving type-3 or 4 reactions, while in the spring, they were only moderately susceptible. Featherston C. I. 1120, Hooded Spring C. I. 716, and Manchuria B44 are examples. Several varieties that gave type-3 or 4 reactions in the winter gave readings as low as 2+ or 2 in the spring. Bohemia C. I. 204 was occasionally slightly more susceptible in spring than in winter. Horsford C. I. 507 and Malting C. I. 1129 are typical of the many varieties that gave type-3 or 4 reactions in both winter and spring tests to both physiologic races.

The reactions of Goldfoil C. I. 928, Hanna C. I. 906, Bohemia C. I. 204 and Palestine C. I. 939 to races 6 and 7 were interesting because these were strains of the same botanical variety of *Hordeum distichon*. Bohemia was very susceptible to both races, Palestine was very resistant to both races, but Hanna and Goldfoil were highly resistant to race 6 and very susceptible to race 7.

On seedling leaves of susceptible plants so-called "green islands" were sometimes observed, especially during the spring tests. The leaf tissue under and immediately adjoining the mildew pustules remained green, while the remainder of the leaf became yellow. In the winter, infected leaves of susceptible varieties succumbed more quickly to the mildew, and "green islands" were seldom noted.

TABLE 2.—*Reaction of barley varieties to two physiologic races of powdery mildew, Erysiphe graminis hordei, in greenhouse studies*

Variety	Accession No. <sup>a</sup>	Type of reaction of					
		seedling plants				adult plants	
		to				to	
		P. r. 6		P. r. 7		P. r. 6	
		Winter	Spring	Winter	Spring	Winter	Spring
Abyssinia .....	362	1	0-1	1	1	1	0
Abyssinian .....	1243	1	0-1	1+	1	1	0-1
Albacaete .....	1128	0-1	0	0	0	1	0
Arequipa .....	1256	1-2	1	1+	1	1-2	0
Arlington .....	702	0	0	0-1	0	0-1	0
Black Hull-less .....	666	0-2	0-1	0-1	1-	0-1	0
Black Hull-less .....	1032	0-2	1-1+	1-2	1-2	0-1	
Blackhull .....	878	1	1-	0-1	1	0-1	0
Bohemia .....	204	3-3+	2-4	3+	3-4	2-3	
Bolivia .....	1257	1	0-1	0-1+	1	1	0
Callas .....	2440	2	1-2	3		0-1	0
Chevron .....	1111	0-1	0	0	0	0	0
Chilean C .....	1432	0-1	0-1	0	0-1	0	0
Chilean D .....	1433	0-1	0-1	0	0-1	0	0
Chinerme .....	1079	0	0	0	0	0	0
Coast .....	276	0-1	0-1	0-1	0-1	0	
Common Chile .....	663	1	0	0-1	0-1	0-1	
Consul .....	1061	1-	1-	1	1-	0-1	0
Duplex .....	2433	0	0	0	0	0	0
Featherston .....	1120	3-4	3	4	3	3-4	1+
Goldfoil .....	928	0	0	3-4	3+	0	0
Hanna .....	906	0	0	3-4	3+	0	0
Hanna .....	966	1	1	1	1-	1-	
Heil's Hanna 3 .....	682	3-4	3	3-4	3+	2-3	0
Hooded Spring .....	716	3-4	3	3-4	3	4	0-1
Horsford .....	507	3-4	3	3-4	3	3	0
Horsford .....	610	3-4	3-4	3-4	3-	3	0
Horsford .....	877	4-	4-	3-4	3	3-4	2-
Juliaea .....	1114	1-3	1	1-3	1-2	1-2	0
Kwan .....	1016	0-1	0-1	0-1	0-1	0-1	0
Lion .....	923	1-2	1-2	1-2+	1-	1	0
Luth .....	972	0-1	0-1	0-1	0-1	0-1	0
Lynch .....	919	0-1	0	0-1+	0	0	0
Malting .....	1129	3-4	3	4	3	3-4	0-2+
Manchuria .....	2330	3-4	3-4	4	3	4	0-2
Meeknos Moroc .....	1379	3-3+	2+	3-4		2-3	0-1
Monte Cristo .....	1017	0	0	0	0	0	0
Nepal .....	475	1-2	1	1	1	1	0
Nepal .....	595	0-1+	1	0-1+	1	0-1	0
Oderbrucker .....	940	3-4	2-3	3-4	3	3-4	1-2
Oderbrucker .....	957	3	3-	4		4	1-2
Palestine .....	939	0-1	1-	1	1	1	0
Peru .....	653	1-2	0-1	1-1+	1-	0-1	
Peruvian .....	935	0-1+	1-	0-1+	0-1	1	0-1
Peruvian .....	1131	0-1	0-1	1	0-1	1	0
Purple Nepal .....	1373	1-	1-	1	1-	0-1	0
Quinn .....	1024	3	3	4		3+	1-2
Sulu .....	1022	0-1	0	0-1	0	0-1	0-1
.....	1021	0-1	0-1	0	0-1	0-1	0
Turkestan .....	711	0-1	0-1	1	1-	1	0

TABLE 2.—(Continued)

Variety	Accession No. <sup>a</sup>	Type of reaction of					
		seedling plants				adult plants	
		to				to	
		P. r. 6		P. r. 7		P. r. 6	
		Winter	Spring	Winter	Spring	Winter	Spring
Turkestan .....	1080	0-1	0-1	0-1	0-1	1	0
	1347	3-3+	2-3	3	3	3	0
	2329	1	1	1	0-1		0
	2416	1	0-1	0-1	0-1	1	
	2444	0	0	0	0	0	
California Feed .....	B59	3	3	3-4	2	2	
Coast .....	B121	1	1	1+	1-	1	
Colseas .....	B97	1	1	1+	1-		
Lion .....	B33	3-4	2-4	3-4	3-	2+	
Locride .....	B86	0-1	0-1	0-2	1+	1-2	
Manchuria Sel. C163 .....	B40	3-4	3-4	3-4	3	3-4	
Manchuria Sel. C164 .....	B41	3-4	3-	3-4	3	3	
Manchuria Minn. 184 .....	B43	3-4	3	3-4	3	3	
Manchuria .....	B44	3-4	3	3-4	3	3-4	
O. A. C. 21 .....	B61	3-4	2-4	3-4	3	3-4	
Orge Fourragère .....	B102	0-1	1	1	1	0-1	
Orge 4th .....	B100	3-4	3-4	4-	3-	2	
Orge 14 J .....	B101	3-4	2-4	3-4	1-3	1+	
Psaknon .....	B81	0	0-1	0	0	0	
Sahara .....	B95	1	1	1-2	1-	1-1+	
Smooth Awn × Manchuria...	B35	3-4	3	3-4	2+	3-4	
Smooth Awn × Manchuria...	B36	3-4	3	4	2	3	
Smooth Awn × Manchuria...	B37	3-4	2-3	4	3	3	
Smooth Awn × Manchuria...	B38	3-4	2-3	4	3	3-4	
Virginia Hooded .....	B6	3-4	3-4	4	3	2-3	
No. 22 .....	B69	0-1	0-1	0	0-1	1	
No. 305 .....	B85	1-3	1-3	2-3	1-2	1	
Glabron .....	M781	3-4	3-	3	3	3-4	
Manchuria .....	M785	3-4	2-3	3+	3-	3-4	
Minsturdi .....	M784	0-1	1	1	1	1	0
Sacramento .....	M777	0-1	1-	0-1	1-	0-1	
Spartan .....	M788	3	3-	3-4	3	3	
Svansota .....	M786	3-4	2-3	3-4	3	3	0
	M756	0	0	0	0	0	
	M757	0	0	0	0	0	

<sup>a</sup> Numbers in this column *not* preceded by a letter are C. I. numbers of the Division of Cereal Crops and Diseases, United States Department of Agriculture. Numbers preceded by B are the numbers under which the varieties were received from W. L. Waterhouse (13, p. 880). Numbers preceded by M are accession numbers of E. B. Mains.

### Reactions of Adult Plants

Adult plants of certain varieties also were inoculated at two seasons of the year in greenhouse studies. In these tests physiologic race 6 was used for inoculum.

The reactions of 83 varieties were obtained in the winter investigations (Table 2). Readings were made in March when the plants were in the late

shooting or heading stages. Varieties, resistant in the seedling stage, were resistant also in the adult stage. Necrosis of leaf tissue, very prevalent on seedling plants of such varieties as Kwan C. I. 1016, Sulu C. I. 1022, and Minsturdi M784, was much reduced or entirely lacking. Instead, only a browning of the host tissue was apparent. Chlorosis and flecking were more pronounced on some varieties than in seedling tests. Almost all varieties, moderately or highly susceptible as seedlings, were equally susceptible in the adult stage. On many varieties, such as Malting C. I. 1129 and several strains of Manchuria, which gave a type-3 or 4 reaction, the mildew was abundant on leaf sheaths and beards, as well as on the leaf blades. In many instances the heads were well past flowering, yet the beards were heavily mildewed. A few varieties, susceptible as seedlings, were slightly more resistant in the adult stage, giving reactions of type-2 to 3. Mecknos Moroc C. I. 1379 is an example. It was further noted that heavily mildewed plants were delayed in coming to full maturity, and the seeds in the heads were greatly reduced in number.

In another experiment, conducted with a limited number of adult plants, readings were made in November and early December, a mixture of races

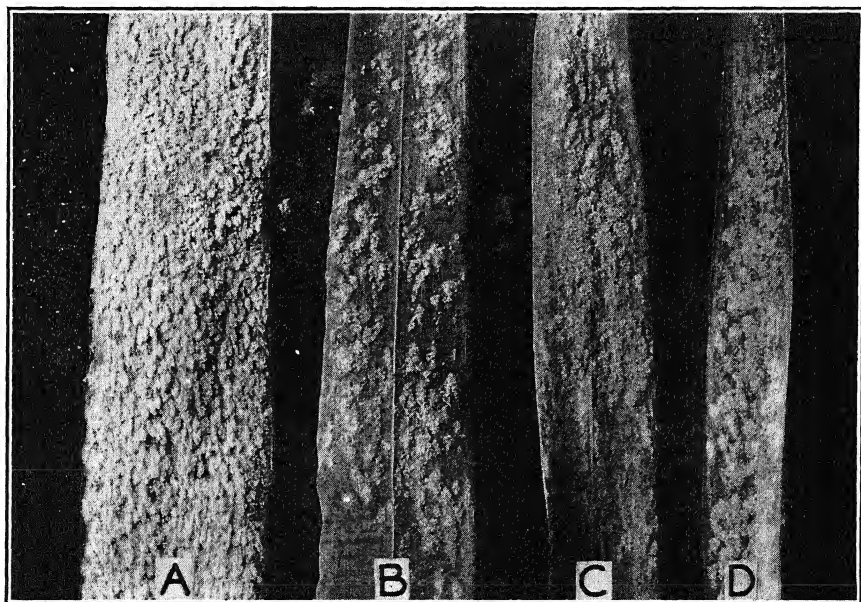


FIG. 2. Upper leaves of adult plants of four susceptible varieties of barley inoculated in winter tests in greenhouse studies with a mixture of races 6 and 7 of powdery mildew.  $\times 1$ . A. Flag leaf of Manchuria M785, type 4. B. Oderbrucker C. I. 940, third leaf from top of plant, type 3. C. Virginia Hooded B6, third leaf from top of plant, type 3. D. Smooth Awn  $\times$  Manchuria B36, flag leaf, type 3.



6 and 7 being used for inoculum. Results were obtained that again indicated that, given certain environmental conditions, adult barley plants are as susceptible to mildew as are seedling plants of the same varieties (Fig. 2).

In the spring series most of the varieties were in the shooting or heading stages when exposed to mildew conidia. Readings were made during the first 2 weeks of May, and the data in table 2 records the reaction noted at the time of full expression of the mildew infection. As shown in table 2, all the 48 adult varieties tested in the spring were more or less resistant. Varieties, highly resistant in the seedling stage, were equally resistant in the adult stage. Some of the varieties, showing type-3 or 4 reactions as seedlings, were highly resistant. Horsford C. I. 610 is typical. Others, such as Oderbrucker C. I. 940 and Quinn C. I. 1024, which were susceptible in the seedling stage, gave reactions ranging from 1 to 2 on the upper leaves. A slight chlorosis of leaf tissue accompanied the slight to moderate development of mycelium on many of these varieties.

#### Reactions of Wild Species of *Hordeum*

The following wild species of *Hordeum* were used in these experiments: Seven collections of *Hordeum murinum* L., 6 collections of *H. gussoneanum* Parl., 2 collections of *H. nodosum* L., and single collections of *H. pusillum* Nutt. and *H. jubatum* L.

The plants were first inoculated in the usual manner with physiologic race 6 in winter of 1934-1935, when they were in the 3-leaf stage. For a period of 4 weeks following this first inoculation they were constantly exposed to inoculum from heavily mildewed barley varieties that were nearby on the greenhouse bench. At no time during this period did any signs or symptoms of the disease appear on the wild species of *Hordeum* thus inoculated.

#### INHERITANCE OF RESISTANCE

##### Svansota M786 × Hanna C. I. 906

Both Svansota and Hanna are 2-rowed barleys. In the seedling stage in the winter, Svansota is very susceptible (type 3-4) to race 6, whereas Hanna C. I. 906 is highly resistant (type 0). The reactions of the  $F_2$  seedlings were obtained in studies made in December, 1933.  $F_3$  progeny tests were conducted in February, 1935.

In the  $F_2$  test 98 plants gave a type-4 reaction, 228 a reaction of 1 or 1-2, and 118 a reaction of 0. This approximates a 1 : 2 : 1 ratio of 111 : 222 : 111, the deviations for the 3 classes being - 13, 6, 7 and  $\text{Dev.} \div \text{P.E.}$  2.11, 0.84, 1.14.

$F_3$  progeny tests were made of 25 to 35 plants derived from each of 262 of the  $F_2$  plants. Forty-eight susceptible  $F_2$  plants proved to be homozygous for susceptibility. Sixty-five of the 67 individuals, classed as highly resistant (type 0) in the  $F_2$ , bred true for high resistance. The progenies



in the other two lines segregated. The misclassification of these  $F_2$  plants is not surprising when the closeness of reaction of the two resistant classes is considered. Ten of the 147  $F_2$  plants, classified as type 1 or 1-2, gave  $F_3$  progenies with only 0 reaction. The plants in the remaining 137 lines segregated.

Of 3983  $F_3$  plants from the 139 segregating lines 1039 gave a type-4 reaction, 2011 a reaction of 1 or 1-2, and 933 a reaction of 0. This approximates a 1:2:1 ratio of 995.75:1991.50:995.75, the deviations for the three classes being 43.25, 19.50, -62.75 and  $\text{Dev.} \div \text{P.E.}$  2.34, 0.92, 3.40.

The results obtained in the  $F_2$  and verified by tests of  $F_3$  lines indicate that the resistance of Hanna C. I. 906 to physiologic race 6 in the seedling stage is due to a single Mendelian factor. Apparently, the marked resistance of Hanna is not completely dominant. Heterozygous individuals, although resistant, are less so than the Hanna parent.

#### Featherston C. I. 1118 $\times$ Goldfoil C. I. 928

Featherston is a 6-row and Goldfoil a 2-row variety. Goldfoil is highly resistant (type 0) to race 6. The reaction of Featherston to race 6 was not obtained. The reactions of  $F_2$  seedling plants were obtained in May, 1934, and those of  $F_3$  lines in February, 1935. Physiologic race 6 of the mildew was used for inoculum.

In the  $F_2$  test 37 plants gave a type-3 or 4 reaction, 70 a reaction of 2-3, and 38 a reaction of 0, 1, or 2. This closely approximates a 1 susceptible:2 intermediate:1 resistant ratio of 36.25:72.50:36.25, the deviations for the 3 classes being 0.75, -2.50, 1.75 and  $\text{Dev.} \div \text{P.E.}$  0.21, 0.62, 0.50.

Tests of approximately 25 to 35  $F_3$  plants from seed of each of the  $F_2$  plants showed that very few of the  $F_2$  plants were wrongly classified. One of them, classed as susceptible (3-4) in the  $F_2$ , produced offspring that segregated; while one plant, classed as intermediate (2-3) in the  $F_2$ , gave rise only to susceptible individuals. Six  $F_2$  individuals, classed as resistant (0-2) and that had given type-2 or 2- reactions, should have been placed in the intermediate group in classifying the  $F_2$  since the progeny in the  $F_3$  lines from these plants segregated. These errors in classification are not surprising when the closeness of reaction of the  $F_2$  plants is taken into consideration.

It is noteworthy that in the  $F_2$  test, conducted in late spring in the greenhouse, heterozygous individuals gave a reaction of type 2-3, intermediate between resistance and susceptibility. In the winter, however, heterozygous  $F_3$  plants were more resistant and gave type-1-2 reactions. Plants, homozygous for resistance and for susceptibility, showed greater stability. The individuals, homozygous for resistance, however, were not all so resistant as the resistant parent, indicating that other factors may play a part in modifying the resistance.

Of the 2466  $F_3$  plants from 76 segregating lines, 614 gave reactions of type 3 or 4, 1216 a reaction of 1-2, and 636 a reaction of 0 or 0-1. This approximates a 1:2:1 ratio of 616.5:1233:616.5, the deviations being -2.5, -17, 19.5 and  $\text{Dev.} \div \text{P.E.}$  0.17, 1.01, 1.34.

The  $F_2$  and  $F_3$  results indicate that the resistance of Goldfoil C. I. 928 to physiologic race 6 is governed by one main factor.

The 145  $F_2$  plants whose seedling reactions to mildew had been ascertained were grown to maturity and classified for 2-row and 6-row arrangement. One group included 111 2-row individuals and the other contained 34 6-row plants, which approximates a 3:1 ratio. This is in agreement with studies of other investigators (25, 3, 4) who have found a single-factor difference between 2-row and 6-row forms, the 2-row condition being dominant.

A combination of the data concerning number of rows per head with that obtained for the inheritance of mildew reaction gives the results shown in table 3. The  $F_2$  plants, heterozygous for resistance, are placed in the resistant class, since the heterozygous  $F_3$  individuals from these plants tended, in the winter tests, more toward resistance than susceptibility.

TABLE 3.—*Inheritance of mildew reaction and row number in the  $F_2$  of a cross between Featherston C. I. 1118 and Goldfoil C. I. 928*

Item	2-row		6-row		Total
	Resistant	Susceptible	Resistant	Susceptible	
Observed .....	84	27	24	10	145
Expected .....	81.56	27.19	27.19	9.06	
Deviation .....	2.44	-0.19	-3.19	0.94	
Probable error .....	4.03	3.17	3.17	1.97	
$\text{Dev.} \div \text{P.E.}$ .....	0.61	0.06	1.01	0.48	

These data indicate a close approximation to the results that would be expected if mildew reaction and row number are inherited independently.

#### Arequipa C. I. 1256 $\times$ Horsford C. I. 610

The variety Arequipa is awned and Horsford is hooded. Seedling plants of Arequipa give a reaction of type 1-2 to race 6, in the winter. Under similar conditions Horsford gives a type-3-4 reaction. The data concerning the reactions of both  $F_2$  and  $F_3$  seedlings were obtained during the winter months in greenhouse studies.

When the  $F_2$  plants were classified all types of reaction from 1 to 4 were encountered. One hundred and forty-two of the plants gave a reaction of type 3 or 4, and 382 a reaction of 1 or 2. This closely approximates a 1:3 ratio of 131 susceptible:393 resistant, the deviation being 11, and

Dev.  $\div$  P.E. 1.64. The results indicate that the expression of resistance is due to a single main Mendelian factor.

In order to verify the results obtained in the  $F_2$  generation,  $F_3$  progenies of 25 to 35 plants derived from each of 122  $F_2$  plants were studied. Four of the  $F_2$  plants were apparently erroneously classified. Twenty-five of the 122  $F_2$  plants were found to be homozygous for susceptibility, 34 were homozygous for resistance, and 63 were heterozygous for resistance-susceptibility. Of the 1881  $F_3$  plants from the 63 segregating lines, 491 gave a reaction of type 3 or 4, and 1390 a reaction of 1 or 2. This approximates a 1 : 3 ratio of 470.25 susceptible : 1410.75 resistant, the deviation being 20.75, and Dev.  $\div$  P.E. 1.64. The results obtained in the  $F_2$  and  $F_3$  indicate that one main Mendelian factor governs the inheritance of the resistance of Arequipa C. I. 1256 to race 6 of the mildew.

The 122  $F_2$  plants, studied for mildew reaction in the seedling stage, were matured and classified for type of lemma, hooded or awned. Some of the plants bore heads that were hooded like the Horsford parent. Some had the hoods elevated on short awns. The latter probably were heterozygous for the hooded character. Both types were recorded as hooded. Other plants were awned like the parent, Arequipa. Eighty-nine plants were hooded and 33 were awned, which is a close approximation to a 3 : 1 ratio. As shown here and as proved by other investigators (25, 3, 4), the hooded character is dominant. A single pair of factors evidently is involved.

Since the genotypic constitution of the 122  $F_2$  plants classified for mildew reaction was indicated by the  $F_3$  progeny tests, the  $F_3$  results may be used for classifying the  $F_2$ . When these data are combined with those involving the hooded and awned characters, results are obtained as given in table 4.

TABLE 4.—*Inheritance of mildew reaction and lemma character in the  $F_2$  of a cross between Arequipa C. I. 1256 and Horsford C. I. 610*

Item	Hooded		Awned		Total
	Resistant	Susceptible	Resistant	Susceptible	
Observed .....	75	14	22	11	122
Expected .....	68.63	22.87	22.87	7.63	
Deviation .....	6.37	-8.87	-0.87	3.37	
Probable error .....	3.70	2.91	2.91	1.80	
Dev. $\div$ P.E. ....	1.72	3.05	0.30	1.87	

Apparently mildew reaction is inherited independently of lemma character.

#### DISCUSSION

As early as 1902 Marchal (17) showed that the powdery mildew of grasses (*Erysiphe graminis* DC.) comprised a number of physiologic varieties, each

specialized on different grass genera. In 1930 Mains and Dietz (15) reported that the *Hordei* variety contained physiologic races distinguished by the differential reactions of varieties of barley. In the present study the occurrence of 2 additional physiologic races has been demonstrated. These have been designated as races 6 and 7. In order to separate race 6 from race 1, another barley variety, Heil's Hanna 3 C. I. 682, has been added to the list of 4 differentials used by Mains and Dietz. In 1934 Honecker (9) also reported the occurrence of 2 new physiologic races of *Erysiphe graminis hordei* in Germany. Since the reactions of 2 of the 5 differentials to the physiologic races isolated by Honecker are not known these 2 German races have not been included in table 1 of the present report, nor have they been designated by numbers.

The discovery of physiologic races of barley mildew should help to account for some of the discrepancies in the results obtained by early workers. For instance, Marchal (17, (1902)) and Salmon (28) obtained slightly different results when certain species of *Hordeum* were inoculated. It is probable that these investigators worked with different physiologic races. In the present study 5 wild species of *Hordeum* were entirely resistant to race 6. Marchal (17, (1902)) reported positive results on *Hordeum murinum* and *H. jubatum*, and Reed (20) obtained positive results on the first leaves only of *H. nodosum*. It also may be that strains of the host, differing in susceptibility, were used in the various investigations.

In the present study the reactions of varieties resistant in the seedling stage were exceedingly stable despite the fact that certain varieties were slightly more resistant in spring than in winter. Likewise, seedling plants of susceptible varieties were found to give reactions in spring very similar to those recorded in winter for these same varieties.

The effect of environmental factors on the reactions of adult barley in inoculation experiments with mildew in winter and late spring in the greenhouse is well defined. In winter, under conditions of low light intensity and lower temperatures, the varieties susceptible in the adult stage were fully as susceptible as in the seedling stage. Adult plants of varieties resistant as seedlings gave reactions very similar to those recorded in the seedling stage. In spring, however, the adult varieties, susceptible during winter and spring in the seedling stage, were moderately to highly resistant. Adult plants of varieties resistant in the seedling stage were often more resistant than seedlings of the same varieties. Instead of giving 0-1 reactions, as did many of the seedlings, the reactions were type 0. This pronounced resistance of adult plants in the greenhouse in spring may be due to increased light or higher temperatures acting on the host-parasite complex.

Graf-Marín (7) states that he never observed mildew on the leaves of mature barley plants, although seedlings of the same variety developed the

disease. He attributed the resistance of the leaves of adult barley to the fact that the haustoria of the fungus were unable to penetrate the thicker cuticle and walls of the epidermal cells of "old leaves". It should be noted that his conclusions were based on results obtained with only one variety grown in the greenhouse in the spring. It is conceivable that had Graf-Marín inoculated adult plants of this variety in winter, infection would have resulted, as it did on adult plants of susceptible varieties in the investigation here reported. In this connection it also might be noted that Miss Mackie (11) found no excessive morphological differences between leaves of resistant and susceptible barley varieties.

Several investigators (32, 33, 6, 8, 10) have observed that certain growing conditions are more favorable for barley-mildew development and the resistance or susceptibility of the host may be somewhat modified. From the results obtained in the present studies it also is evident that the reactions of some varieties may be modified by environmental factors. This is especially true of adult plants of susceptible varieties as already mentioned. With seedling plants, however, the changes were for the most part not pronounced. This conclusion is supported by the results obtained in the genetical studies.

The "green island" phenomenon, observed in spring on leaves of susceptible varieties in the seedling stage, has been noted by several investigators. Graf-Marín (7) noted "green islands" on barley leaves that had almost been killed by ultraviolet light. Reed (22) mentioned the occurrence of green areas infected with mildew. The yellowing of barley leaves beyond the infected green regions was suggestive of the secretion of toxic substances by the mildew (11). Miss Rice (24) has given a good discussion of the occurrence of this phenomenon in the rusts. In the writer's experiments the "green islands" were occasionally noted on a few leaves that died from causes other than mildew infection. Such "green islands" were very small. The best examples were observed on seedling leaves of some of the susceptible varieties in the spring tests. These leaves generally were fairly heavily infected with mildew and the "green islands" gradually became prominent as the leaf yellowed.

The genetical studies with seedling plants demonstrate that resistance is a definitely inherited character. The resistance of the 3 resistant parent plants studied, Hanna C. I. 906, Goldfoil C. I. 928, and Arequipa C. I. 1256, was shown in each case to be due to a single main Mendelian factor. The resistance of Hanna was incompletely dominant, the heterozygous individuals being somewhat less resistant than Hanna and homozygous resistant segregates. The same was true for Goldfoil. In crosses where Arequipa was the resistant parent, heterozygous individuals were not so clearly differentiated. The resistance of Arequipa is not so stable as that of Hanna or Goldfoil and is more easily varied by environmental conditions and probably is responsible

for the intergradation of the homozygous resistant and heterozygous groups. Honecker (9) in his studies during the winter months also was able to distinguish 3 classes that gave approximately a 1:2:1 ratio. During the summer months, reactions were so modified that individuals, heterozygous or homozygous for resistance, could not be distinguished. In his studies Dietz (5) found susceptibility dominant.

#### SUMMARY

The reactions of 85 varieties of barley in the seedling stage in greenhouse studies to 2 new physiologic races of *Erysiphe graminis hordei* are given. These 2 races are designated as physiologic races 6 and 7. To the list of 4 differential barley varieties used by Mains and Dietz (15), a fifth variety, Heil's Hanna 3 C. I. 682, was added.

Five wild species of *Hordeum* were highly resistant to physiologic race 6 in tests conducted in winter in the greenhouse.

Seedling barley plants, in winter and spring, have shown no marked changes in reaction at these two seasons to either race 6 or race 7. Certain varieties are slightly more resistant in spring.

Tests of varieties in the adult stage in greenhouse studies, have shown that such barley plants, in spring, are more resistant to mildew than seedling plants of the same varieties. Adult plants in winter are fully as susceptible as seedling plants of the same varieties.

Studies of  $F_2$  and  $F_3$  of 3 crosses between resistant and susceptible plants indicate that resistance or susceptibility to physiologic race 6 of barley mildew in the seedling stage is inherited in definite Mendelian proportions. (1) In the cross, Svansota M786  $\times$  Hanna C. I. 906, a single pair of factors is involved. Resistance is incompletely dominant. (2) In the cross, Featherston C. I. 1118  $\times$  Goldfoil C. I. 928, a single pair of factors is involved. Resistance is incompletely dominant. The reactions of heterozygous individuals were altered by environmental factors. Independent inheritance of the factor pairs for resistance versus susceptibility, and 2-row spikes versus 6-row spikes is indicated. (3) In the cross, Arequipa C. I. 1256  $\times$  Horsford C. I. 610, a single pair of factors is involved. Resistance is dominant. Independent inheritance of the factor pairs for resistance versus susceptibility and hoods versus awns is suggested.

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# STUDIES ON REPRESENTATIVE STRAINS OF TOBACCO-MOSAIC VIRUS<sup>1</sup>

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## INTRODUCTION

Several investigators have described symptoms produced by one or more strains of tobacco-mosaic virus (1, 2, 3, 5, 6), but no effort has been made to define or to describe the range of symptoms produced by any considerable number of strains in different species. Demonstration and description of the extensive range of symptoms obtainable by infection with various strains of tobacco-mosaic virus will, it is believed, aid in arriving at a better understanding of the relationships between virus strains.

Fifty-five distinct strains of tobacco-mosaic virus were studied. These were Johnson's tobacco virus 1 (5) and 54 strains derived directly or indirectly from this virus. Symptoms produced by each strain varied in some respect from those produced by any of the other strains. The purpose of this report is to show the extent of the symptom range exhibited by these strains of tobacco-mosaic virus and to report on the differences in infectivity and resistance to heat shown by the strains of virus.

## MATERIALS AND METHODS

Fifty-one of the 54 strains were obtained by making single pin-puncture inoculations to healthy tobacco plants from bright yellow spots on tobacco, tomato, or *Nicotiana sylvestris* Spegaz. and Comes plants infected with tobacco-mosaic virus (3, 4). The other 3 were obtained from various sources. One was the masked-symptom strain isolated by Holmes (2). The second appeared in one of 10 tobacco plants that had been inoculated with virus from a single necrotic lesion on a leaf of *N. langsdorffii* Weinm.<sup>2</sup> previously inoculated with tobacco-mosaic virus. The third was isolated in the following manner: A diluted sample (1:50) of tobacco-mosaic virus was rubbed over 3 large expanded leaves on each of 123 *N. sylvestris* plants. Within a few days many chlorotic lesions and, in addition, a small number of circular necrotic lesions appeared on the 369 inoculated leaves. In the hope that a virus capable of producing only necrotic lesions on leaves of *N. sylvestris* would be isolated, several of the necrotic lesions were cut out and transferred, by the glass-spatula method (3), to leaves of healthy *N. sylves-*

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<sup>2</sup> This plant referred to in previous publications (3, 4) as *N. langsdorffii* Schrank.

*tris* plants. Many yellow and a few necrotic primary lesions appeared on the inoculated leaves. Virus samples from single necrotic lesions were again transferred as before and both yellow and necrotic lesions were produced. After making a series of 5 such transfers, the virus causing only necrotic lesions on *N. sylvestris* was obtained. It was similar to the green-mosaic virus isolated by Kunkel (6) and by Smith (7) from plants affected by aucuba mosaic.

For the most part, the host plants used were *Nicotiana tabacum* L. var. Turkish, *N. sylvestris*, and *N. glutinosa* L. In a few instances plants of *Lycopersicon esculentum* Mill. also were used. Data on symptoms were based on observations of a succession of inoculated plants studied during a period of several years. All plants were grown in 4-inch or 6-inch unglazed clay pots or in shallow wooden flats. The greenhouse temperature varied from about 68° F. to about 90° F., with an average temperature of about 72° F.

The rubbing method of inoculation was used in all routine transfers of virus. The efficacy of this method was augmented by the use of finely ground carborundum (No. 320) for the transfer of virus strains having low infectivity. Other special methods of inoculation used have been described previously (3).

All thermal inactivation and infectivity studies were based upon inoculations made with freshly expressed juice. Except in the case of the masked-symptom strain virus, samples were obtained only from leaves showing symptoms. The upper leaves of plants infected with the masked-symptom strain were used. The tissue was ground in a sterile food chopper and the juice expressed by pressing the pulp by hand in a cheesecloth bag. Temperature exposures of juice from diseased plants were made by placing small test tubes (1 × 10 cm.) containing 3 cc. of juice in a large insulated water bath warmed by a hand-regulated electric heater, and constantly agitated by means of an electrically driven stirring rod. The water bath was held within 0.5° C. of the desired temperature.

#### CHARACTERISTIC SYMPTOMS PRODUCED BY 12 REPRESENTATIVE VIRUS STRAINS

Twelve strains of tobacco-mosaic virus were selected for detailed study. The symptoms produced by these 12 strains are representative of those caused by the 55 strains studied. Table 1 presents a brief description of the symptoms produced on 3 host species, *Nicotiana tabacum* var. Turkish, *N. sylvestris*, and *N. glutinosa*. The following statements indicate the outstanding characteristics of each of the strains, as well as the characteristics that distinguish the various strains from ordinary tobacco-mosaic virus.

*Green-mottling distorting-type strain (Johnson's tobacco virus 1 (5)):* Symptoms of this virus, commonly known as ordinary tobacco-mosaic virus,

TABLE 1.—*A brief description of the symptoms produced by 12 strains of tobacco-mosaic virus on 3 host plants*

Virus strain	Tobacco		<i>Nicotiana sylvestris</i>		<i>Nicotiana glutinosa</i>
	Primary lesions	Systemic infection symptoms	Primary lesions	Systemic infection symptoms	Primary lesions
Ordinary tobacco mosaic	Faint yellow	Green mottling	Faint yellow	Green mottling	Large <sup>a</sup>
101	Bright yellow	Erect leaf and yellow mottling	Necrotic	Systemic necrosis	Ordinary <sup>b</sup>
108	Bright yellow	Yellow mottling	Necrotic	None	Ordinary
501	Faint yellow	Green mottling	Necrotic	None	Ordinary
302	Bright yellow	Yellow mottling	Yellow	Yellow mottling	Ordinary
111	Yellow	Yellow mottling	Large yellow	Yellow spotting	Ordinary
9	Faint yellow	Light yellow spotting	Necrotic	None	Ordinary
104	Small yellow	Yellow spotting	Small yellow	Yellow spotting	Minute
502	Faint yellow	Mild green mottling	Faint yellow	Mild green mottling	Ordinary
Masked-symptom	Invisible	Invisible	Invisible	Invisible	Small <sup>c</sup>
3	Yellow	None	Yellow	None	Ordinary
14	Necrotic	None	Necrotic	None	Ordinary

<sup>a</sup> 1 to 2 mm. in diameter 5 days after inoculation.

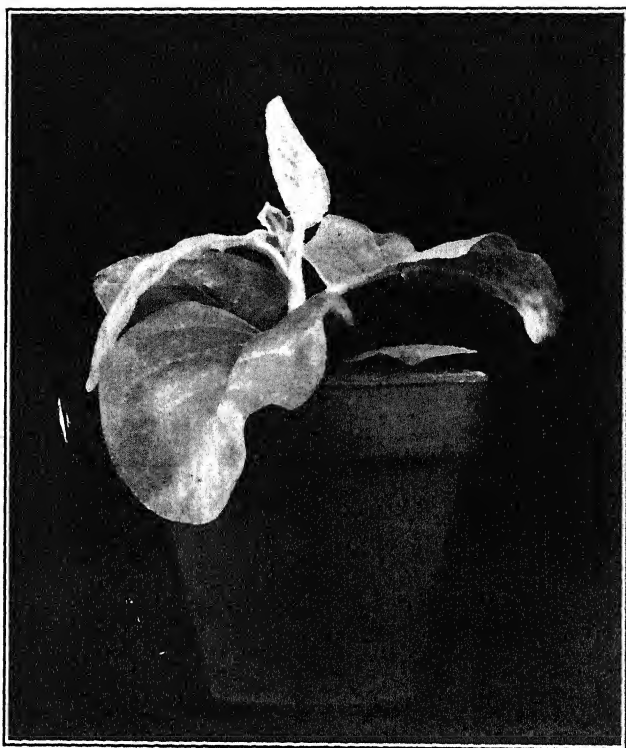
<sup>b</sup> Like lesions produced by tobacco-mosaic virus.

<sup>c</sup> Slightly smaller than lesions produced by tobacco-mosaic virus.

have been completely described many times, and no need is felt for further discussion of them here. All other strains mentioned in this report were derived directly or indirectly from this virus strain. Figure 2, A, shows symptoms of this virus on tobacco.

*Yellow-mottling distorting-type strain:* In tobacco this virus strain, listed as No. 101, causes a yellow mosaic, which reaches the growing point and produces mottling and distortion of young leaves at the tip of the plant. From 1 to 2 days after the clearing-of-veins symptom is first observed, 1 or sometimes 2 of the leaves that show this symptom assume a marked upright or erect position in which they remain for 1 or more days. Figure 1 is from a photograph of a plant showing this peculiar response. This strain kills young plants of *Nicotiana sylvestris*.

*Yellow-mottling aucuba-type strain:* This virus, designated as No. 108, produces symptoms essentially like those described for aucuba mosaic (7). It causes yellow mottling in tobacco and necrotic primary lesions in *Nicotiana sylvestris*. Figure 2, C, shows the symptoms produced on tobacco.



Photograph by J. A. Carlile

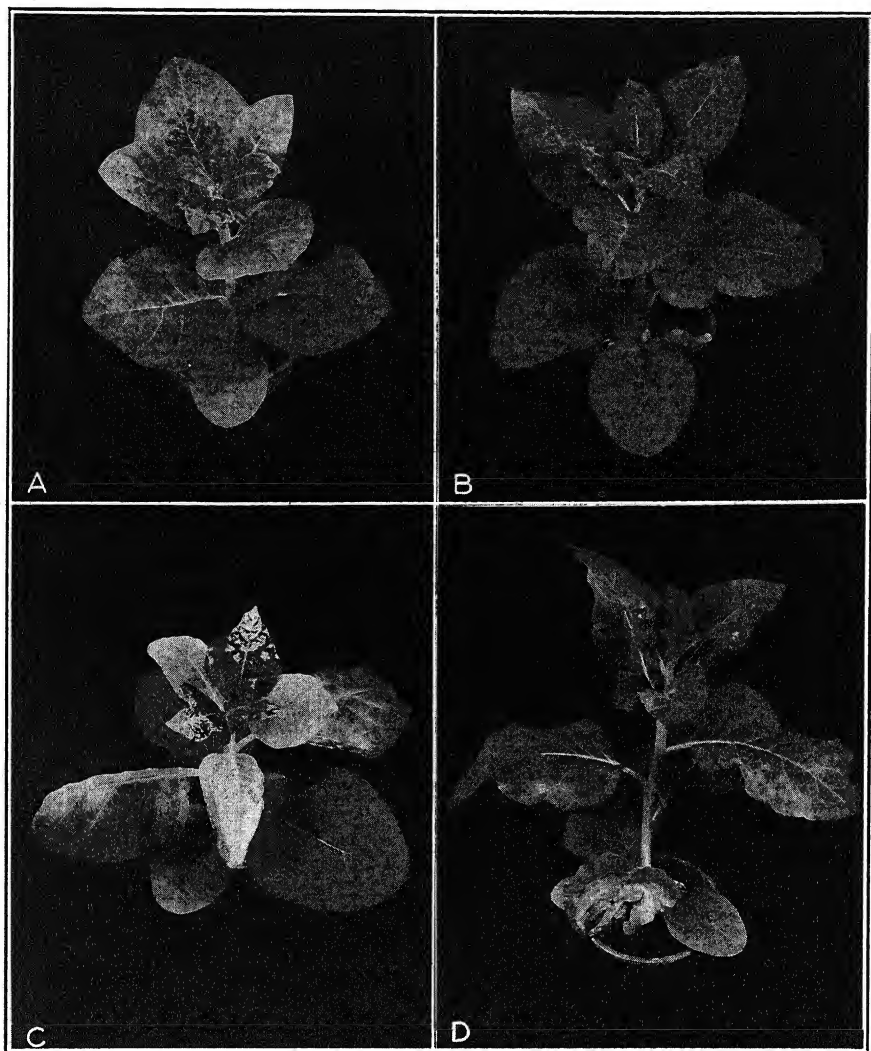
FIG. 1. Symptoms of strain No. 101 on tobacco. The uppermost leaf in the photograph shows the upright position assumed by one of the vein-clearing leaves 5 days after inoculation. This leaf assumed its normal position 3 days after the photograph was taken.

*Green-mottling aucuba-type strain:* Virus No. 501 produces symptoms essentially like those described by Kunkel (6) and Caldwell (1) for the green aucuba-mosaic virus. It causes green mottling in tobacco and necrotic primary lesions in *Nicotiana sylvestris*.

*Yellow mottling-type strain:* Virus No. 302 produces a typical yellow mosaic, but is slightly less invasive than the viruses causing the yellow mosaics mentioned above. It produces yellow mottling in both tobacco and *Nicotiana sylvestris*.

*Yellow spotting-type strain:* This virus, designated as No. 111, is moderately slow-moving in tobacco plants, causing large yellow spots with centers of deep green instead of mottling symptoms. It differs sharply from the other virus strains in that it never produces vein-clearing symptoms in tobacco (Fig. 2, D).

*Light green spotting-type strain:* This virus, listed as strain No. 9, causes light green spotting instead of green-mottling symptoms in tobacco. Several weeks after inoculation, inconspicuous brownish necrotic spots appear on some of the older leaves.



Photograph by J. A. Carlisle

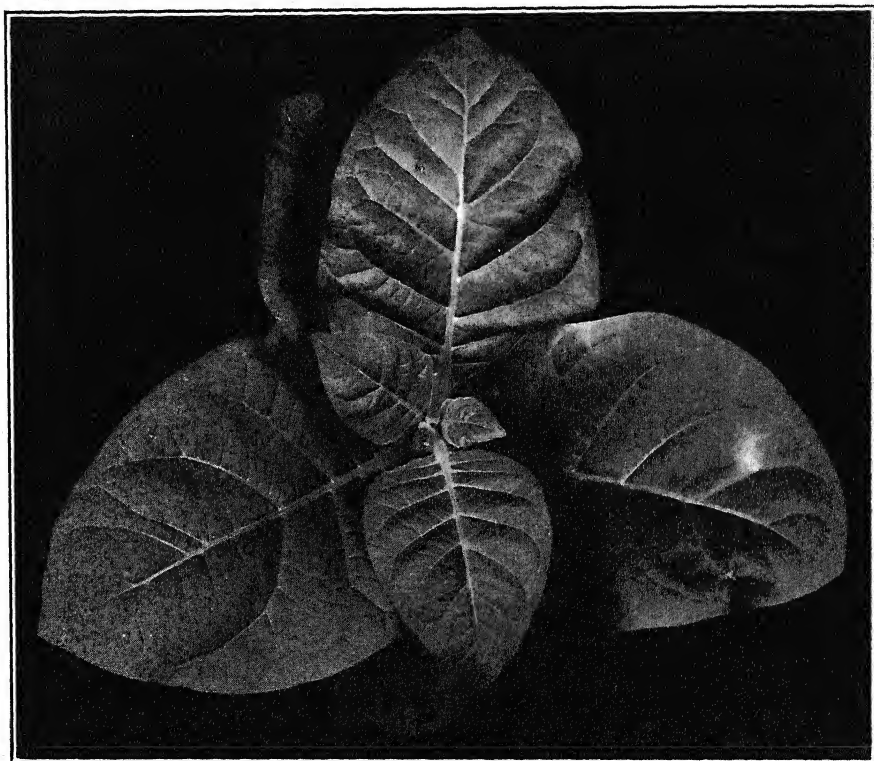
FIG. 2. Symptoms produced on tobacco by 4 strains of tobacco-mosaic virus. A. Ordinary tobacco-mosaic virus. B. Strain No. 104. C. Strain No. 108. D. Strain No. 111.

*Mild yellow spotting-type strain:* Virus No. 104 causes a systemic yellow spotting on tobacco, as is shown in figure 2, B, and can be readily distinguished from all other strains by the minute necrotic lesions it produces on *Nicotiana glutinosa*. Figure 6 illustrates 2 leaves of *N. glutinosa*, one inoculated with ordinary tobacco-mosaic virus and the other with strain No. 104.

*Non-distorting green-mottling strain:* Virus strain No. 502 causes a mild green mottling of tobacco, the symptoms of which are identical with or simi-

lar to those described by Holmes (2) under the name of "mottling strain."

*Masked-symptom strain (Holmes(2))*: Ordinarily sets of tobacco plants inoculated with this virus strain can not be distinguished from sets of non-inoculated plants. It causes systemic infection without the production of visible symptoms.

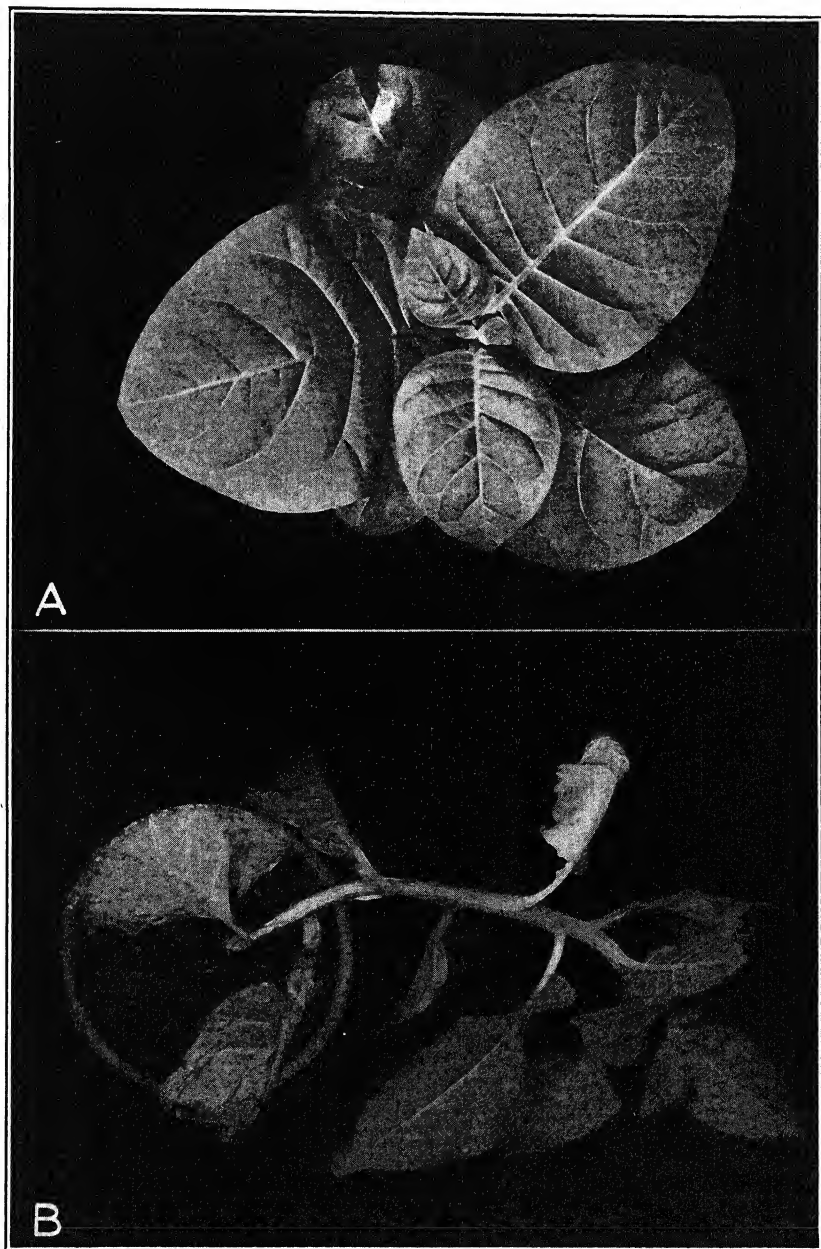


Photograph by J. A. Carlile

FIG. 3. Symptoms of strain No. 3 on tobacco. Twelve days after inoculation: no symptoms other than yellow primary lesions have appeared.

*Slow-moving yellow-spotting-type strain*: Virus No. 3 is very slow moving, causes pale yellow spots on tobacco leaves, as shown in figure 3, never causes mottling, and is of low infectivity. The yellow primary lesions tend to enlarge along the midveins. In tobacco, yellow oak-leaf patterns occasionally appear on leaves near the tips of infected plants.

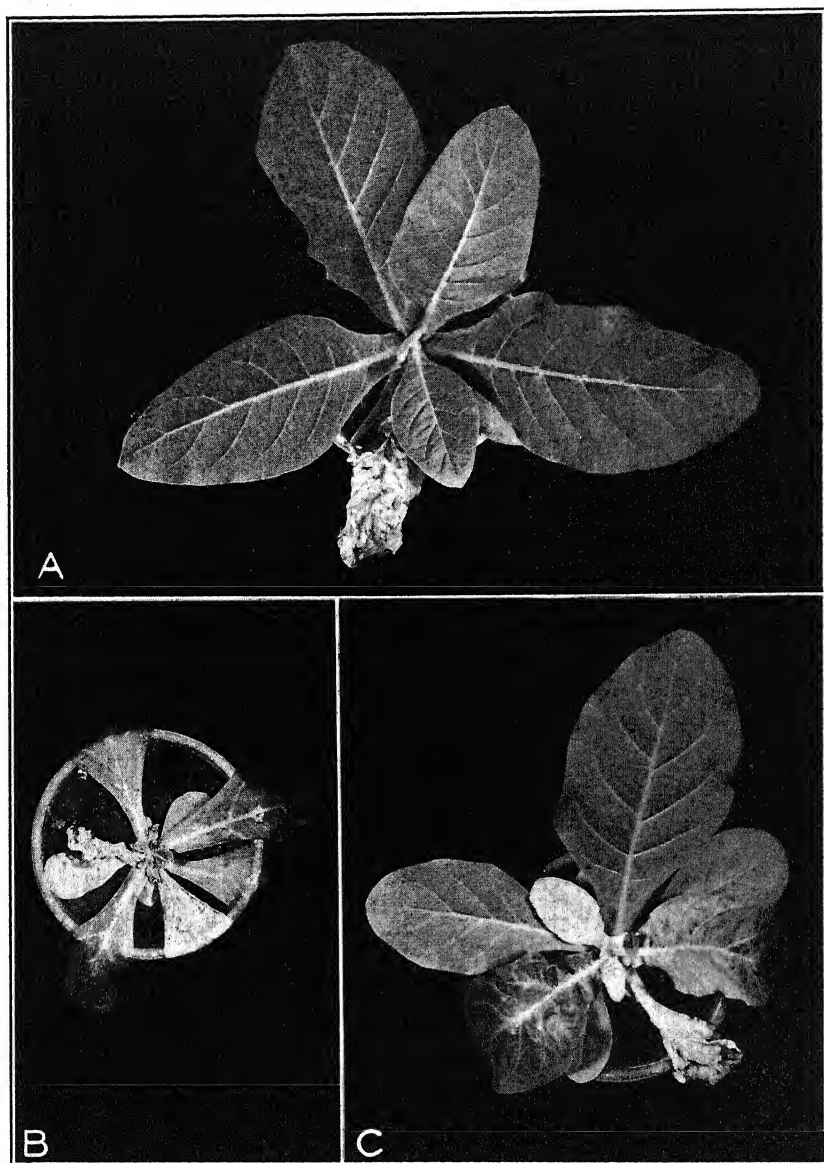
*Necrotic-type virus strain*: This virus strain, designated as No. 14, produces necrotic primary lesions on tobacco, as shown in figure 4, A and B, never becomes systemic, and is of low infectivity. In tomato and tobacco the necrosis sometimes spreads down the petiole of infected leaves and into the stem, causing the plants to fall over.



*Photograph by J. A. Carlile*

FIG. 4. Symptoms of strain No. 14 on tobacco. A. Twelve days after inoculation: no symptoms other than a necrotic primary lesion have appeared. B. Two months after inoculation: necrosis has extended down into the stem and the plant has fallen over. No symptoms have appeared in the tip leaves.





Photograph by J. A. Carlile

FIG. 5. Symptoms of 3 isolations on *Nicotiana sylvestris*. A. Strain No. 108 produced only necrotic primary lesions. The inoculated leaf subsequently collapsed and died. B. Strain No. 101 produced a severe chlorosis and the plant finally died. C. Strain No. 302 produced a yellow-mottling disease. The inoculated leaf died. All plants were of the same age and were inoculated at the same time.



## RANGE OF SYMPTOMS

The 55 strains of tobacco-mosaic virus fall into 3 general classes when the symptoms appearing on tobacco are studied: (1) Those that produce a generalized systemic infection, as in the case of ordinary green and yellow mosaic strains. (2) Those that produce spotting without mottling. Viruses in this group cause vein-clearing only when heavy inoculations are made on several leaves at the same time. (3) Those in which systemic infection, in the usual sense, does not occur. Plants infected with viruses of this group occasionally show oak-leaf infection patterns on one or more of the lower leaves. The group includes slow-moving yellow-type and necrotic-type strains.

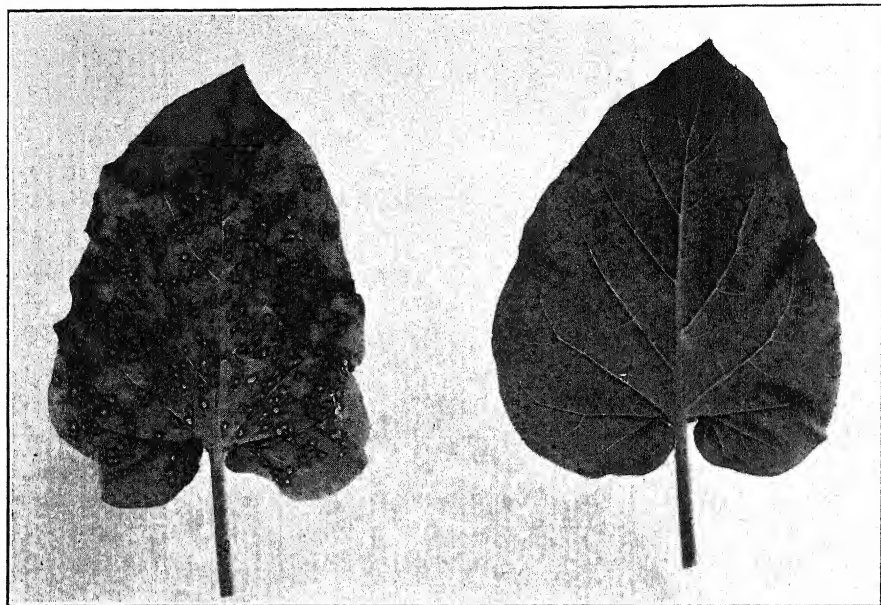
The reaction of *Nicotiana sylvestris* to certain strains is very striking, and for that reason this species was included as a test plant. In general, 3 types of primary lesions are produced: they may be yellow, similar to those obtained with ordinary tobacco-mosaic virus; they may be necrotic; or they may be invisible. Systemic infection in *N. sylvestris* produces green and yellow mottling diseases, systemic necrosis, and masked-symptom infection. Certain virus strains invariably kill young *N. sylvestris* plants. Usually such strains cause necrotic primary lesions. Figure 5 shows 3 types of reactions obtained in this plant.

The appearance of the necrotic primary lesions produced by tobacco-mosaic virus on leaves of *Nicotiana glutinosa* is well known. With 2 exceptions, all strains of virus that were tested produced necrotic lesions that appeared identical with those produced by ordinary tobacco-mosaic virus. The masked strain produced slightly smaller lesions than are caused by ordinary tobacco-mosaic virus, whereas strain No. 104 produced lesions that were much smaller, as shown in figure 6. These minute lesions required a longer time for development than do the lesions caused by tobacco-mosaic virus.

## INFECTIVITY EXPERIMENTS

Throughout the course of these investigations it was noted that virus strains show great variations in the ease with which they are transmitted. A number of strains were found to be as easily transmitted as ordinary tobacco-mosaic virus, the high infectivity of which is well known, whereas others were considerably less easily transferred, and still others were transmitted to healthy plants only with difficulty. A high percentage of infections was obtained with the latter strains only through the use of powdered carborundum.

In an attempt to present the range in transmissibility on a numerical basis, several series of experiments were designed in which 2 types of inoculation and 2 species of host plants were used. In the first series of experi-



Photograph by J. A. Carlile

FIG. 6. Necrotic primary lesions on *Nicotiana glutinosa*. Left: Ordinary lesions produced by tobacco-mosaic virus. Right: Minute lesions produced by strain No. 104. Both leaves were of the same age and inoculated on the same day.

ments single pin-puncture inoculations were made from leaves of diseased plants to a small leaf on each of 50 healthy tobacco plants. The number of

TABLE 2.—Number of infected plants obtained by making single pin-puncture inoculations into sets of 50 young tobacco plants with each of the 12 tobacco-mosaic virus strains studied

Virus strain	Test 1	Test 2	Test 3	Test 4	Total
Ordinary .....	19	25	23	28	95
101 .....	19	15	16	18	68
108 .....	8	9	12	10	39
501 .....	14	20	11	10	55
302 .....	6	8	2	2	18
111 .....	0	0	0	0	0 <sup>a</sup>
9 .....	8	12	13	14	47
104 .....	28	25	21	22	96
502 .....	14	16	17	15	62
Masked-symptom ...	16	13	14	14	57
3 .....	0	0	0	0	0 <sup>a</sup>
14 .....	0	0	0	0	0 <sup>a</sup>

<sup>a</sup> In additional tests single pin-puncture inoculations were made to 700 young healthy tobacco plants with each of virus strains 111, 3, and 14. From this number of inoculations 6 plants were infected with strain 111, 1 with strain 3, and 2 with strain 14.

inoculated plants that became diseased varied from almost 50 per cent, in the case of 2 mottling-type strains, to less than 1 per cent, in the case of a slow-moving yellow-spot virus strain. Table 2 shows the number of diseased plants obtained in 4 different tests and the total number of infections obtained in all 4 tests.

A second series of experiments was set up in which various dilutions of the virus strains were rubbed over leaves of *Nicotiana glutinosa* and the resulting number of necrotic primary lesions were recorded. The Latin-square method proposed by Youden and Beale (8) was utilized. Experiments were arranged so that the same dilutions of 5 virus strains were compared in each set of plants. Each virus strain was represented on each plant once and on each leaf position once in every test. The tests were repeated a number of times, and in each repetition a different combination of 5 virus strains was used, the purpose being to test the individual virus strains against as many other virus strains as practicable.

The data from one test are shown in table 3; other tests gave similar results. In this and in other experiments the 2 slow-moving virus strains, Nos. 3 and 14, were not tested against the other virus strains because their low infectivity made it desirable to rub more than 5 test leaves and also to take extra precautions against possible contamination with other virus strains. The figures shown in the cases of strains Nos. 3 and 14 represent the average numbers of lesions that appeared on 25 inoculated leaves.

TABLE 3.—Average numbers of lesions obtained per leaf of *Nicotiana glutinosa* rubbed with various dilutions of the tobacco-mosaic virus strains indicated

Virus strain	Dilutions					
	1: 1 <sup>a</sup>	1: 2	1: 4	1: 10	1: 100	1: 1000
Ordinary .....	290	270	190	91	47	10
101 .....	139	92	84	62	27	5
108 .....	63	53	36	29	13	3
501 .....	63	67	37	28	7	1
302 .....	69	52	56	22	7	1
111 .....	0.4	0.8	0.2	0	0	0
9 .....	71	73	56	25	15	1
104 .....	162	106	106	55	28	2
502 .....	103	113	79	66	34	7
Masked-symptom	121	138	84	69	28	11
3 <sup>b</sup> .....	0.2	0	0	0	0	0
14 <sup>b</sup> .....	0.2	0	0	0	0	0

<sup>a</sup> 1: 1 represents undiluted juice.

<sup>b</sup> Figures for numbers of necrotic lesions obtained by inoculations with virus strains 3 and 14 are based upon 25 different leaf tests. No lesions were obtained with any diluted juice samples.

The experiments demonstrate that the strains of tobacco-mosaic virus vary widely in infectivity. Ordinary tobacco-mosaic virus is more infective than most of the other virus strains tested. It seems clear that the slow-moving strains are least infective.

#### THERMAL INACTIVATION EXPERIMENTS

One of the properties frequently used to characterize certain plant viruses is the thermal inactivation point. As is well known, the thermal inactivation point of tobacco-mosaic virus for a 10-minute exposure is about 90° C. Other plant viruses are inactivated at lower temperatures.

The variability in symptoms produced by various strains of tobacco-mosaic virus suggested the possibility that the virus strains might differ in their resistance to heat. Accordingly, viruses of the 12 strains, the symptoms of which have been described, were subjected to 10-minute exposures of 70°, 80°, 85°, and 89° C. In each experiment 10 viruses were divided into 2 sets of 5 viruses each, the 2 slow-moving strains, Nos. 3 and 14, being tested separately in order to minimize chances for accidental contamination. Juices containing each of the 5 virus strains in 1 test were exposed to the various temperatures simultaneously. Immediately after the temperature exposure, tubes containing the viruses were set in cold water to cool. Inoculations on leaves of *Nicotiana glutinosa* were carried out immediately after cooling. The Latin-square method of leaf arrangement was again used.

Table 4 shows the average numbers of lesions per inoculated leaf obtained with the strains. It is to be noted that lesions were obtained with virus of every strain exposed to a temperature of 80° C. for ten minutes, but that several virus strains apparently did not survive exposures to temperatures beyond that point. These results might be interpreted as indicating differences in thermal inactivation points. However, when results of infectivity experiments are studied it will be noted that the same strains that are inactivated at a low temperature also show low infectivity. The results of these two series of experiments, therefore, indicate that infectivity must be considered in interpreting thermal inactivation of a given virus.

#### DERIVED STRAINS

Plants inoculated with slow-moving virus strains occasionally show mottling or other characteristics of fast-moving strains. When subinoculations are made to healthy plants, fast-moving viruses are obtained. It is difficult to explain the presence of fast-moving strains in the tips of plants inoculated with slow-moving viruses in any other way than by assuming that the new strains arise from the viruses originally introduced into the plants.

Although the symptoms produced on young tobacco plants inoculated

TABLE 4.—Average numbers of necrotic lesions obtained per leaf of *Nicotiana glutinosa* rubbed with samples of tobacco-mosaic virus strains heated for 10 minutes to the temperature indicated

Virus strain	Temperature				
	Unheated	70° C.	80° C.	85° C.	89° C.
Ordinary .....	320	280	270	250	95
101 .....	200	179	75	17	0.6
108 .....	162	110	29	2	0.4
501 .....	250	199	87	11	0.2
302 .....	30	30	2	0.2	0
111 .....	1	0.8	0.4	0	0
9 .....	120	120	36	1	0.2
104 .....	350	350	132	85	46
502 .....	270	230	140	125	30
Masked-symptom	270	185	110	60	7
3 .....	1	0.6	0.4	0	0
14 .....	0.8	0.6	0.6	0	0

with virus from tip leaves of plants showing divergent symptoms seemed similar to those produced by ordinary systemic yellow and green mottling virus, nevertheless it appeared desirable to test these viruses on other host plants. Three of the isolations obtained from the tip leaves of tobacco plants inoculated with the slow-moving necrotic virus (strain No. 14) will be described. When the viruses were transferred to tobacco, 1 strain produced a green-mottling disease and the other 2 produced yellow-mottling diseases without any unusual features. When the viruses were transferred to tomatoes, one produced a green-mottling disease, another a yellow-mottling disease, and the third a severe yellow-mottling disease that was followed by a systemic necrosis which, when moderately young plants were infected, always resulted in death of the plants. The symptoms produced by these 3 derivative strains are presented in figure 7. Thus, from ordinary tobacco-mosaic virus a slow-moving necrotic-type strain was isolated, and from this slow-moving strain on tobacco a strain was derived that produced a fast-moving virus causing systemic necrosis in the tomato. Derivation of such highly destructive strains from relatively nondestructive strains may open the way for studies on the nature of variations in virus strains.

#### DISCUSSION

The outstanding characteristic symptoms of 12 strains of tobacco-mosaic virus on 3 species of host plants is presented in this report. The 12 strains produce symptoms that are representative of the range of symptoms of the 55 strains studied.

The variations in symptoms that were reported are characteristic of

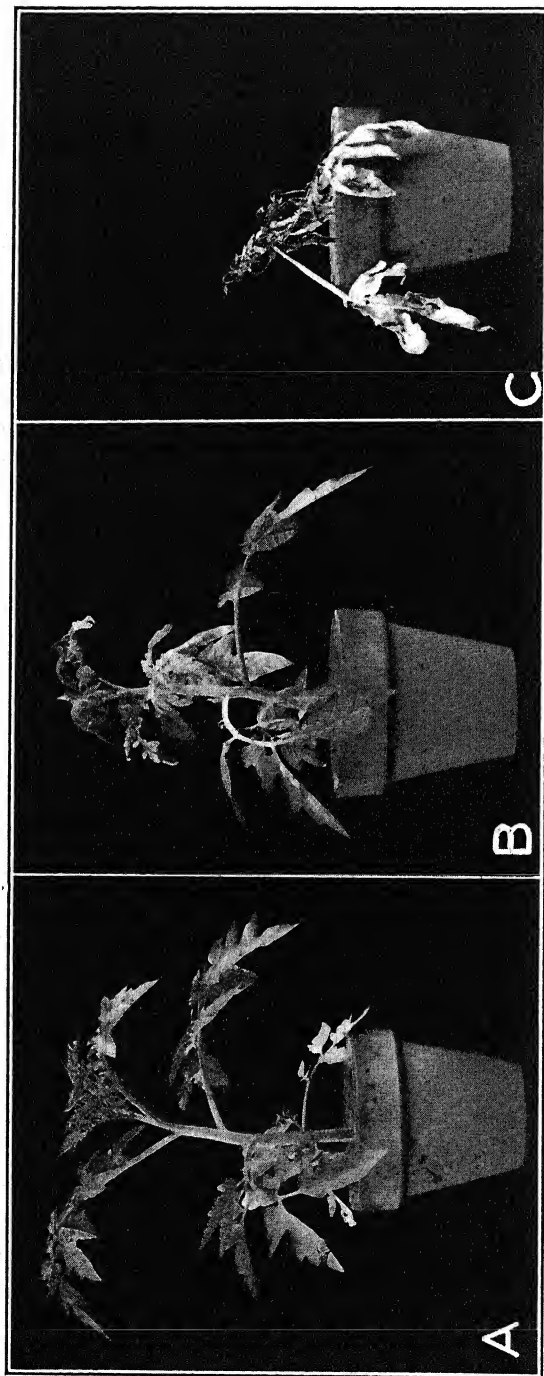


FIG. 7. Symptoms produced on tomato by 3 strains derived from strain No. 14. A. Green-mottling strain. B. Yellow-mottling strain. C. Necrotic strain, which is lethal. All plants were of the same age and inoculated on the same day.

*Photograph by J. A. Cardile*

individual strains. Except in the case of the vein-clearing symptom produced when several leaves were heavily inoculated with spotting viruses, the symptoms produced by a given virus sample were always the same regardless of the dilution of the inoculum or the amount of leaf surface inoculated. No information was gained as to the underlying differences between the virus particles responsible for the variations in symptoms. It can not be said, for instance, whether inability of the slow-moving strains to move rapidly or their low infectivity is due to excessive size, greater toxicity, slower multiplication, or to some other factor. A general correlation was found to exist between rapidity of virus movement and infectivity. All slow-moving strains were difficult to transmit, whereas strains characterized by rapid movement were readily transmissible.

The appearance of mottling viruses in the tip leaves of plants inoculated with slow-moving viruses might seem to indicate that the slow-moving viruses are highly unstable. It will be recalled, however, that most of the strains used in this study have been isolated from plants inoculated with the ordinary tobacco virus, a fact which indicated that variations arise in the rapid-moving viruses also. Slow-moving viruses arising in plants inoculated with rapid-moving viruses have been found only in bright yellow spots. Their inability to move probably prevents their further distribution in the plant tissue. However, when a rapid-moving virus arises from a slow-moving virus, its ability to move into new tissue makes its appearance all the more striking. The derivation of necrotic virus strains from an ordinary widely distributed virus strain presents interesting possibilities. Further work in deriving strains experimentally in this manner may lead to a discovery of the underlying reasons for the variations produced by different virus strains.

#### SUMMARY

Symptoms of 12 tobacco-mosaic virus strains, representative of the range of symptoms produced by 55 strains, are described on tobacco, *Nicotiana glauca*, and *N. glutinosa*. One strain, derived from a slow-moving, necrotic-type strain on tobacco, killed tomato plants. Two strains produced unusually small lesions on leaves of *N. glutinosa*. Single pin-puncture inoculations of some strains to young tobacco plants produced as high as 50 per cent infection, whereas other strains were transmitted in less than 1 per cent of the attempts. Other strains ranged between these two extremes. Infectivity trials using the local-lesion method gave similar results. All strains tested were found to withstand 10-minute exposures to a temperature of 80° C.

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# STUDIES ON "DAMPING OFF" OF CULTIVATED MUSHROOMS AND ITS ASSOCIATION WITH FUSARIUM SPECIES<sup>1</sup>

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## INTRODUCTION

During the course of routine visits to commercial mushroom growers in the south of England it was noticed that many of the beds showed a typical condition known to most of the growers as "damping off." The writer proposes to continue this nomenclature in the ensuing discussion, since it effectively describes the commoner type of condition and, moreover, it is already in use among mushroom growers.

The 3 following characteristic conditions, constantly observed during preliminary investigations on "damping off," were: 1. The beds were full of spawn run, and in many cases the spawn also had run into the casing soil, but little or no mushroom production had taken place.

2. Mushrooms had formed, even to the extent of a good first flush. The crop rapidly degenerated into small, withered, brown, and undeveloped mushrooms. Rotting generally did not take place, the stunted mushrooms remaining indefinitely on the beds in a mummified condition.

3. The crop appeared to be normal and, on occasions, heavy. Sooner or later a big proportion of the mushrooms were found to be dry and pithy inside the stipe, of a rubbery texture, and, generally, the interior of the stipe was quite brown. These mushrooms were of no market value. In the case of the brown variety of mushroom the pilei were burnished and shining in appearance, giving the impression of having been polished, while the normal mushroom showed a matt brown pileus.

No clear demarcation exists between the 3 types of condition, all of which often were found simultaneously on a bed.

In each of the above cited cases it was found that other beds in the same house, under the same growing conditions, and prepared from the same compost, were producing perfect mushrooms. It also often happened that the trouble was localized to a patch, in and around which perfectly healthy mushrooms were formed.

<sup>1</sup> The writer is particularly grateful to Dr. H. W. Wollenweber of the Biologische Reichsanstalt, Berlin-Dahlem, for his unfailing kindness and courtesy in the identification of the many species of *Fusarium* that were isolated during the course of these investigations. He also owes a debt of gratitude to Mr. G. R. Rettew and the Chester County [Pennsylvania] Mushroom Laboratories for their continued help and encouragement, and to the many mushroom growers and fellow mycologists who, from time to time, have so kindly given facilities.

These factors combined with careful observation and attention to cultural details gradually eliminated the possibility of the trouble being due either to pest attack or to faulty watering, bad ventilation, etc.

It also was noticed that "damping off" often would spread rapidly through a bed, and it, therefore, was assumed that a fungus was responsible. With this idea in mind samples from mushroom beds from various commercial growers were examined systematically, special attention being given to the casing soil, since this was generally the only part of the bed that was unsterilized.<sup>2</sup>

Finally, from the material forwarded from Kings Lynn, a *Fusarium* species was obtained and a preliminary inoculation<sup>3</sup> experiment suggested that the real cause of "damping off" of cultivated mushrooms had been found.

Working on this hypothesis, a systematic examination of as many cases of "damping off" as were available was begun and, with surprisingly few exceptions, a *Fusarium* species was found in the casing soil in close proximity to the affected mushrooms.

By far the commonest species were *Fusarium martii* (App. and Wr.) and *Fusarium oxysporum* Schl., and these two species were taken for experimental purposes.

Other species isolated were *F. culmorum* (W. G. Sm.) Sacc., *F. flocciferum* Cda., and several others not yet identified.

#### TECHNIQUE

In all the material examined the following methods were used for determination and isolation of the fungi concerned.

Culture plates were prepared, using the following medium: Cane sugar, 25.0 g.; sodium nitrate, 2.0 g.; potassium chloride, 0.5 g.; potassium phosphate, ( $\text{KH}_2\text{PO}_4$ ) 1.0 g.; magnesium sulphate, 0.5 g.; ferrous sulphate, a trace; agar agar, 30.0 g.; sodium tauroglycocholate, 1.0 g.; distilled water, 1000 cc.

It was found that this medium did not tend to encourage bacterial growth; therefore, the presence or absence of fungus species in the soil surrounding the bases of the stipes of "damped off" mushrooms was determined by plating out soil particles.

If *Fusarium* spp. were present, they grew out from the soil particles and speedily outgrew any bacterial contamination. (Fig. 1, A.) Pure cultures

<sup>2</sup> During the preparation of the compost temperatures of 140-160° F. are often reached. These are far above the thermal death point of *Fusarium* sp. The greater part of the spawn now used is pure culture spawn.

<sup>3</sup> Wood, F. C. "A New Disease of Cultivated Mushrooms." Gard. Chron. XCVII. 243. 1935.

then were obtained by subculturing repeatedly on the following medium: 100 g. soil mixed with 100 cc. water and autoclaved for 30 min. at 10 lbs. pressure. To the filtrate was added 5 per cent malt extract and 2 per cent agar agar.

As regards the inoculation experiments it was obvious from the beginning of the investigation that the following disadvantages would have to be reckoned with: (a) The length of time elapsing between spawning and production is two months. This makes an investigation liable to error, since it necessitates careful control of conditions over a long period. (b) The compost used for growing the spawn is not sterile and cannot be sterilized easily and effectively. (c) The casing soil must be sterilized. (d) The spawn must be uncontaminated. (e) Rigid pest control must be maintained.

The commonest pests to be reckoned with are species of *Hypogastrura*; species of *Tyroglyphus*; *Sciarid* flies; and *Phorid* flies.

After many unsuccessful efforts with inoculation experiments in which results were negated by infestation by *Phorid* fly larvae,<sup>4</sup> the following procedure was adopted: Glass pneumatic troughs, 10 cm. deep and 25 cm. wide, were used. Into these was pressed moist pure culture spawn to a depth of about 6 cms. After allowing a few days to elapse, in order to allow the spawn to run throughout the mass again, the casing soil was applied.

This was good quality clay loam, previously sterilized by heating for 20 min., under steam pressure, to a temp. of 80° C. When cool the soil was broken up and applied to a depth of  $\frac{3}{4}$ –1 in.

The materials in use, therefore, were sterile, since the spawn was broken out directly from the bottle in which it was grown and the soil was sterilized.

This method also eliminated trouble from springtails or mites in the compost. *Sciarid* and *Phorid* flies were prevented from ovipositing by covering the pneumatic trough with a layer of coarse muslin held in position by a rubber band. This method of mushroom growing was found to answer excellently under ordinary laboratory conditions and to produce mushrooms in about 3 weeks.

Inoculations were carried out by spraying the casing soil with a spore suspension of the *Fusarium* species used.

#### DAMPING OFF OF CULTIVATED MUSHROOMS CAUSED BY *FUSARIUM OXYSPORUM* SCHL. AND *FUSARIUM MARTII* APP. AND WR.

##### Symptoms and General Description

In the majority of cases investigated the trouble was noticed by the grower either towards the end of the crop or following a period of heavy picking. Both white and brown varieties of mushrooms have been found to

<sup>4</sup> The species was identified by Dr. Barnes of Rothamsted as *Aphiochaeta halterata*.

be affected, but usually *Fusarium oxysporum* seems to affect the brown variety of mushroom more than the white.

Typically the effect is that mushrooms make their appearance and for a time seem perfectly normal even to maturity. When picked, however, they

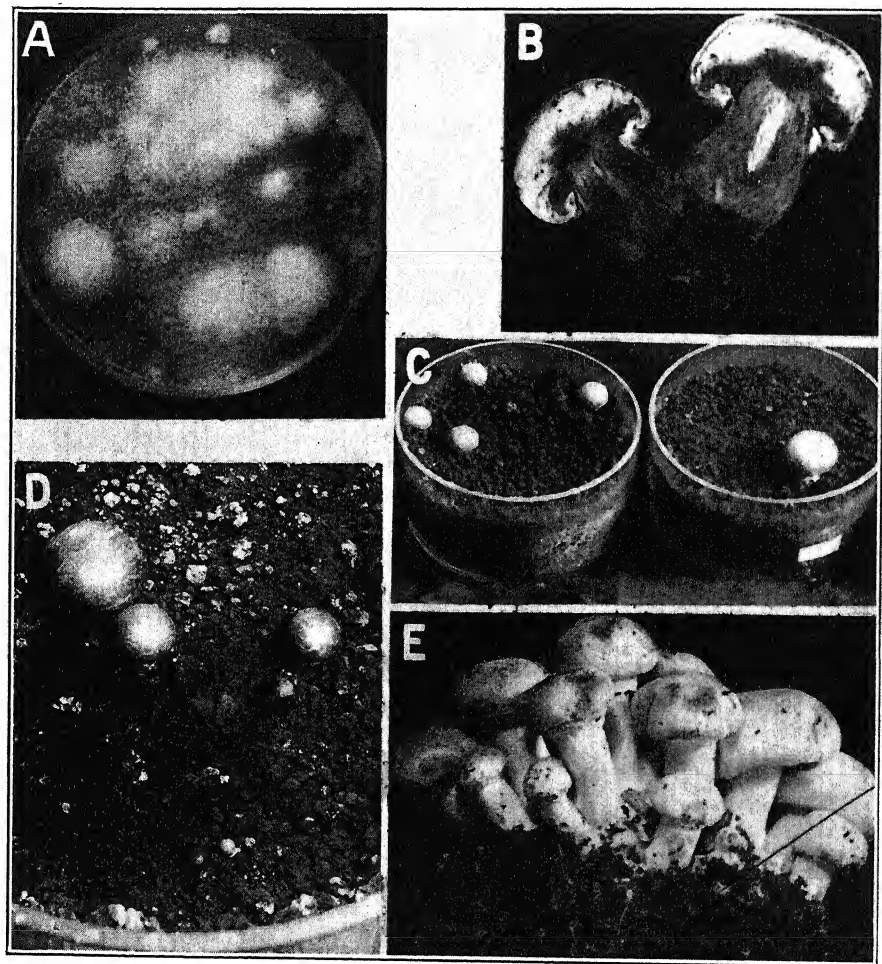


FIG. 1. A. Soil plate showing both *Fusarium oxysporum* and *F. martii*. The large white masses are the mycelium of the former, while the colony on the top left-hand edge of the Petri dish is the mycelium of the latter.  $\times 1$ . B. Longitudinally cut mushrooms showing brown staining caused by *F. oxysporum*.  $\times 1$ . C. Method of growing mushrooms in pneumatic trough. Control dish, left; inoculated dish, right.  $\times \frac{1}{2}$ . D. Effects of inoculating casing soil with a spore suspension of *F. oxysporum*. The upper three mushrooms are normal brown type. Below, on the right, is a smaller mushroom showing the glossy dark brown pileus. Towards the lower edge of the dish are normal and affected buttons side by side.  $\times \frac{1}{2}$ . E. Break of white mushrooms from first flush showing effect of *F. oxysporum* in casing soil.  $\times \frac{3}{4}$ .

show a pithy, withered nature and the interior of the stipe is generally brown, the discoloration showing a gradation in intensity from the base of the stipe to the pileus. (Fig. 1, B). Mushrooms of this type appear in patches among and in close proximity to healthy breaks. Such mushrooms are, of course, valueless for market. If they are picked off, healthy ones may be produced for a time. Eventually, however, the trouble reappears—generally having spread down the beds—and now there is a distinct external difference from the healthy crop. The pileus presents a burnished appearance, totally distinct from the usual matt brown of the healthy mushroom, and has a polished surface. The shade of color is darker than that of the normal mushroom. As before, this stage persists for some time, alternating with healthy mushroom production (Fig. 1, D).

The third stage shows a diminution in size from the normal and, furthermore, a characteristic lopsidedness is noticeable. The stipe being eccentrically placed instead of centrally in relation to the pileus.

This leads to the last stage in which the mushroom never grows beyond the button stage in which stage it becomes mummified and persists on the bed for as long as a month without rotting. (Figs. 2, E and F).

In one or two instances where a heavy infection of *Fusarium* in the casing soil was found, the crop was small and stunted from the outset and never gave promise of being a commercial proposition.

In the case of the white variety of mushroom only two instances of *Fusarium oxysporum* effect have been investigated. In both cases the crop appeared in breaks as in figure 1, E, characterized by very long stems, small pilei, discolored flesh and extremely rapid rotting and decomposition of the breaks.

The effect of *Fusarium martii* infection of the casing soil on mushroom production is much the same, and the above description, with but little modification, will serve. In the case of *F. martii* it seems that the white variety of mushrooms is far more readily affected than the brown and, up to the present time, no case of "damping off" of brown mushrooms has been found in which *F. martii* was responsible.

#### Inoculation Experiments with *Fusarium oxysporum* on Brown and White Varieties of Mushrooms

A. *Brown Variety*.—On July 8 two culture dishes were set up as previously described using brown pure culture spawn and a fairly heavy clay loam casing soil. One dish was used as a control throughout. The other was inoculated by spraying the casing soil with a spore suspension of *Fusarium oxysporum* in distilled water on July 12.

Both dishes were kept under normal laboratory conditions and were watered with sterile distilled water as the spawn ran up into the casing soil.

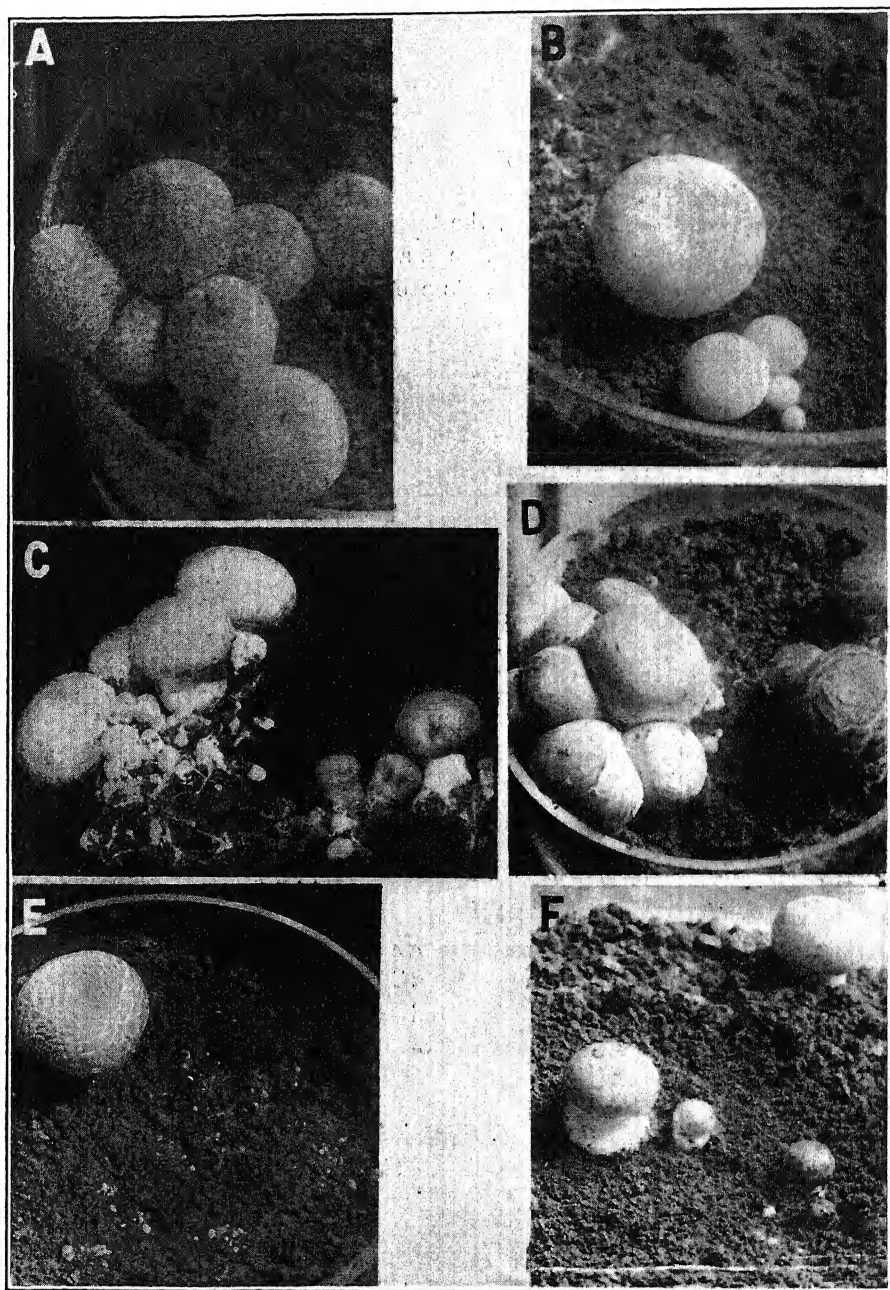


FIG. 2. A and B. *F. oxysporum* and its effect on white mushrooms. A. Control dish. B. Inoculated dish.  $\times \frac{3}{8}$ . C. *F. martii* and its effect on white mushrooms shown on right; control mushrooms on left.  $\times \frac{3}{8}$ . D. Brown control dish, *F. martii*.  $\times \frac{1}{2}$ . E. Inoculation experiment showing healthy mushroom, stunted and mummified "buttons," and patches of *F. oxysporum* showing in the soil.  $\times \frac{1}{2}$ . F. Inoculated dish. Note shrivelled buttons and tiny pips in the foreground. Compare mushrooms with the one in the

On July 26, just over a fortnight from the commencement of the experiment, "pipping up" of the spawn was taking place in the casing soil of the inoculated dish.

The first mushrooms were picked on August 6 (Fig. 1, C, D).

On October 10 (3 months from the beginning of the experiment) soil samples were taken from both the control and inoculated dishes and plates were made as described previously.

In the case of the control dish no *Fusarium* growth was obtained, the plate showing only bacterial contaminations and *Penicillium* sp.

TABLE 1.—Yield in grams of brown and white varieties of mushrooms grown on heavy clay loam casing soil in culture dishes. One was a control series; the other a series of cultures inoculated with a spore suspension of *Fusarium oxysporum*

A. Brown mushrooms		
Date	Yield from control cultures	Yield from inoculated cultures
Aug. 7 .....	105	105.0 <sup>a</sup>
" 16 .....	.....	29.0 <sup>b</sup>
" 19 .....	64	.....
" 29 .....	.....	0.5 <sup>c</sup>
Sept. 2 .....	26	.....
" 16 .....	20	23.0
" 19 .....	.....	.....
" 26 .....	.....	26.5 <sup>d</sup>
" 27 .....	12	8.5
Oct. 7 .....	3	13.0
	Totals ..... 230	205.5
B. White mushrooms		
April 6 .....	38.3	.....
" 7 .....	56.0	.....
" 9 .....	23.6	.....
" 18 .....	Fresh sterile soil added $\frac{1}{2}$ in. deep	Fresh sterile soil added $\frac{1}{2}$ in. deep
" 22 .....	17.2	One small button visible
" 30 .....	29.6	20.0
May 7 .....	.....	5.5 <sup>e</sup>
" 15 .....	17.7	56.5
" 16 .....	60.0	24.3
" 17 .....	.....	30.4
	Totals ..... 254.4	136.7

<sup>a</sup> Many small shriveled pips present.

<sup>b</sup> Flesh not discolored, but leathery and of withered texture.

<sup>c</sup> All were stunted buttons; all brown and none exceeding  $\frac{1}{8}$  in. diam.

<sup>d</sup> Buttons now beginning to show "polished," dark brown appearance.

<sup>e</sup> Mushrooms very small, dwarfed and withered; interior of stipes stained brown.



In the case of the inoculated soil a very heavy growth of mycelium was obtained which eventually was proved to be *Fusarium oxysporum* Schl.

B. *White Variety*.—Inoculation experiments were carried out along the lines indicated, using white pure culture spawn. The experiment was set up on Feb. 20, 1936, and one dish was inoculated by spraying the sterile casing soil with a spore suspension of *Fusarium oxysporum*.

The first production was seen on the control dish on March 26 (Fig 2, A and B).

Comparing results it is seen that the ratio  $\frac{\text{Control production}}{\text{Inoculation production}} = \frac{1}{.88}$  in the case of brown mushrooms and  $\frac{1}{.54}$  in the case of white mushrooms.

These results agree with those observed among commercial growers.

In all cases of *Fusarium oxysporum* infection of the casing soil and brown mushrooms (provided the crop develops under reasonably good conditions), the trouble seldom becomes apparent until the conclusion of crop growth, when the virility of the spawn is beginning to weaken. In cases where *F. oxysporum* infection of the soil is present with white mushrooms the crop generally is doomed to failure at the outset. It would appear, therefore, that the brown type of cultivated mushroom shows resistance to "damping off" by *F. oxysporum*.

#### Inoculation Experiments with *F. martii* on Brown and White Varieties of Mushrooms

A. *Brown Variety*.—This experiment was set up on Feb. 20, 1936. One dish was inoculated with a spore suspension of *F. martii* on March 19 (Table 2).

B. *White Variety*.—Inoculation experiments also were carried out, using the other commonly isolated *Fusarium* species from cases of "damping off," viz., *F. martii* App. and Wr.

The production ratio of  $\frac{\text{Control dish production}}{\text{Inoculated dish production}}$  in the case of the brown variety of mushrooms is  $\frac{1}{.93}$  and in that of the white is  $\frac{1}{.48}$ .

This is consistent with observed results in mushroom houses, as *F. martii* is exceptionally bad in beds of white mushrooms, while it seldom causes trouble in mushroom houses spawned with brown spawn.

#### DISCUSSION

From the results of the inoculation experiments it will be seen that there is a discrepancy in the weight of mushrooms produced in noncontaminated soil and that obtained from soil inoculated with *Fusarium* spp. This difference in weight is not strikingly great but it should be remembered that



TABLE 2.—Yield, in grams, of brown and white varieties of mushrooms grown on casing soil in culture dishes. One was a control series, the other a series of cultures inoculated with a spore suspension of *Fusarium martii*

A. Brown mushrooms		
Date	Yield from control cultures	Yield from inoculated cultures
March 21 .....	31.7	
" 30 .....	.....	3 mushrooms and a few small buttons
April 2 .....	.....	20.6 <sup>b</sup>
" 4 .....	.....	22.5
" 6 .....	120.0 <sup>a</sup>	23.0, a few pips, withered
" 7 .....	13.0	.....
" 9 .....	.....	18.0, mushrooms perfect
" 18 .....	Fresh sterile soil added	Fresh soil added
" 29 .....	17.5	56.0
" 30 .....	14.0	35.8
May 1 .....	29.0	.....
" 15 .....	25.0	39.7
" 16 .....	.....	19.1
	Totals .....	234.7
	250.2	
B. White mushrooms		
Aug. 12 .....	7.5	.....
" 13 .....	5.0	.....
" 20 .....	7.0	.....
" 21 .....	36.0	.....
" 24 .....	1.0	8.75
" 30 .....	6.5	.....
" 31 .....	2.5 <sup>c</sup>	10.5
Sept. 9 .....	9.5	21.0
" 20 .....	8.0	.....
	Totals .....	40.25
	83.0	

<sup>a</sup> See figure 2, D and F.

<sup>b</sup> Mushrooms were perfect.

<sup>c</sup> See figure 2, C.

nothing like normal production is possible under the growing conditions that were perforce adopted.<sup>5</sup>

The supply of food material represented by the moist spawn is very small as compared with the fresh compost available in a newly prepared mushroom bed. Therefore, the production of the control dish is very far

<sup>5</sup> The market value also must be considered, since, although the mushrooms with withered stems or with "brassy" caps weigh well, they are, of course, utterly worthless from a commercial standpoint.

short of being normal. This is shown very well in the weight and size of the mushrooms themselves, which, at maturity, were probably only a third of the weight and size of mushrooms produced on a proper mushroom bed, albeit they were perfectly healthy.

The purpose of the experiment was, however, to prove that the presence of *Fusarium* spp. in the casing soil would give rise to disease symptoms in mushrooms exactly similar to those obtained in cases of "damping off"; and this end was achieved, as hitherto described.

#### SUMMARY

The continual association of "damping off" of cultivated mushrooms and the presence of a species of *Fusarium* in the casing soil was noted:

Species associated with "damping off" symptoms were *Fusarium oxysporum*, *F. martii*, *F. culmorum*, *F. flocciferum*, *F. redolens*, *F. sambucinum*, *F. sambucinum* form 6.

The commonest species were *Fusarium oxysporum* and *F. martii*. These were, therefore, studied in detail.

Inoculation experiments, involving *Fusarium*-infested casing soil and its effect on mushroom production, are described.

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# HISTOLOGICAL AND CYTOLOGICAL STUDIES OF ETHYL MERCURY PHOSPHATE POISONING IN CORN SEEDLINGS

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## INTRODUCTION

The use of dusts in which the active ingredient is ethyl mercury phosphate produces a characteristic malformation of the seedlings of corn and other cereals. As described by Crozier,<sup>1</sup> the plumule and radicle of treated seeds fail to elongate rapidly, but they enlarge greatly in diameter. The malformation exhibits all gradations from nearly normal to a short, compact,

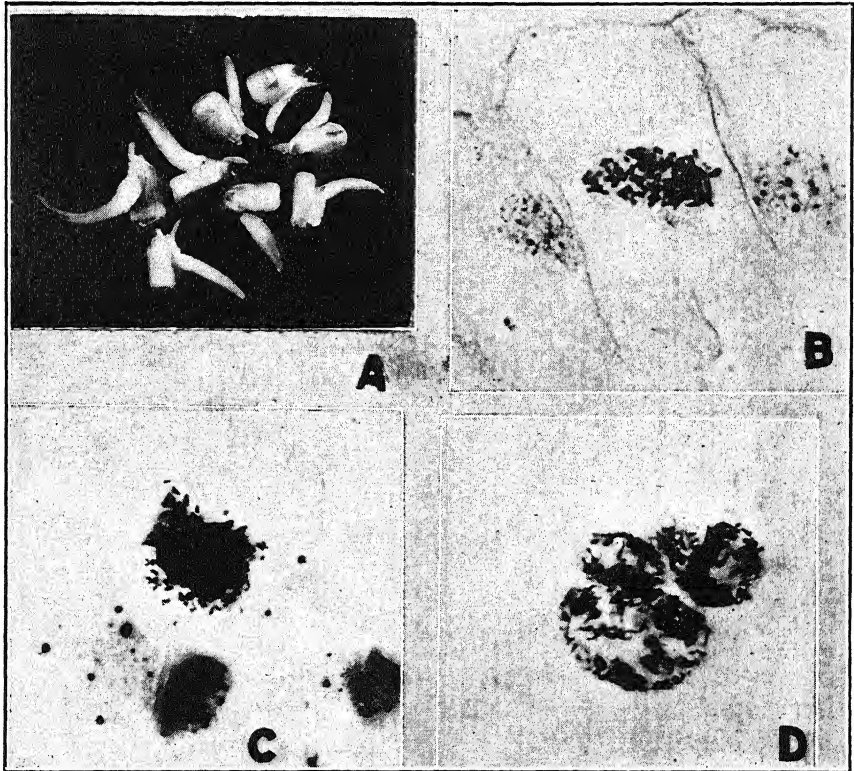


FIG. 1. A. Corn seedlings, two weeks after soaking seed corn in a 1:1500 solution of New Improved Ceresan. B-C. Metaphase figures of giant nuclei, showing large chromosome complement. The anaphase split can be seen in many chromosomes.  $\times 700$ . D. Late prophase of the four nuclei in a single cell. The nuclei differ in size and chromosome complement.  $\times 700$ .

<sup>1</sup> Crozier, W. Abnormal germination in dusted wheat. *Phytopath.* 24: 544-547. 1934.

fusiform condition. (Fig. 1, A.) The present study was undertaken to determine the anatomical and cellular changes associated with these malformations in seedlings of corn.

#### MATERIALS AND METHODS

A collection of seedlings grown after treatment by the usual dry dust method, was obtained from C. S. Reddy. Subsequent collections of material for sectioning were obtained from seedlings grown as follows: kernels of yellow-dent corn were soaked in a 1:1500 solution of New Improved Ceresan for 24 hours, drained, and germinated in moist chambers. All of the seedlings exhibited the malformation. Small sections were removed from the desired regions of seedlings and killed in the following fluid: 1 per cent chromic acid, 20 cc.; 1 per cent acetic acid, 75 cc.; formalin (40 per cent), 5 cc. An interval of 48 hours suffices for killing and hardening. No washing in water is required before dehydration in grades of acetone. The tissues of the coleoptile are sufficiently soft to section easily after the usual method of infiltration in paraffin. Pieces from the region of the scutellum, which include areas of brittle tissue, were dehydrated in a "butyl alcohol-acetone" series, essentially similar to the well known "ethyl alcohol-butyl alcohol" series. After imbedding by this process, sectioning is facilitated by soaking the mounted blocks of tissue in water at 35° C. for about 24 hours. The slides were stained with "Hemalum." Sections containing lignified cells were counter-stained with safranin.

For the study of cytological changes, root tips were used. Seedlings were grown in moist chambers, moistened with water, until the seminal roots reached a length of about 1 cm. The seedlings were then transferred to dishes containing blotting paper moistened with a 1:1000 solution of Ceresan. The growing roots, which curved down into the solution, developed pronounced terminal swelling and ceased apical elongation. These tips were cut off and prepared for sectioning. Sections were stained in iron-hematoxylin.

#### OBSERVATIONS

In corn seedlings grown from nontreated seed, the leaf primordia and apical meristem of the coleoptile have the structure characteristic of meristematic tissues. The cells are small, polygonal, compactly arranged, and of uniform size (Fig. 2, E). These cells are strictly uninucleate, and the nuclei are of uniform size. In older leaf primordia the cells are larger, more vacuolate, and begin to show evidence of differentiation into tissues, but the uninucleate condition is obvious. The surface outlines of the young leaves are smooth and nonlobate (Fig. 2, B-E).

Seedlings from treated seeds exhibit varying degrees of distortion of

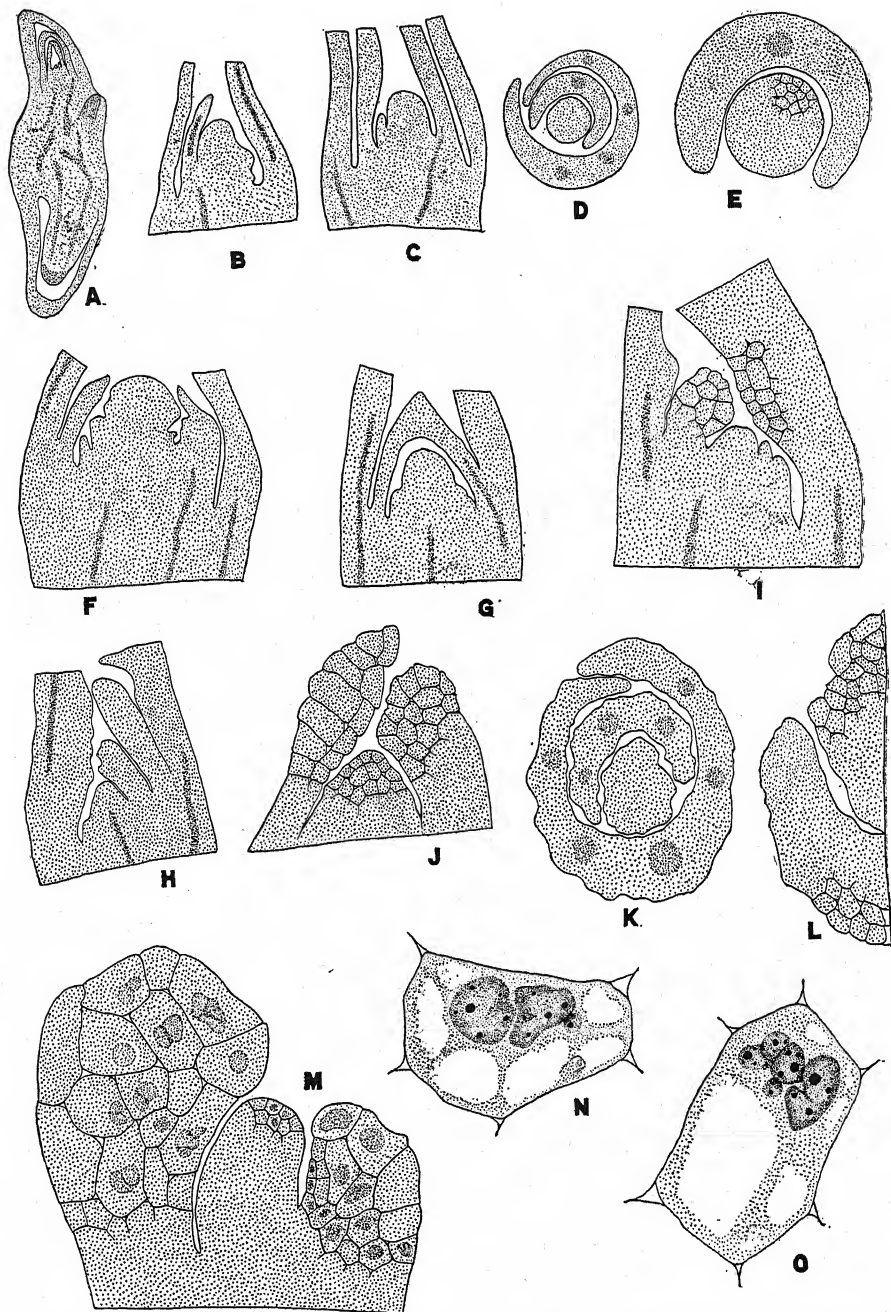


FIG. 2. A. Median section of normal embryo of corn, removed from scutellum, showing the sheathed apical meristems. B-C. Growing point of normal stem, showing leaf primordia. D. Transverse section near tip of normal growing point. E. Same section as D, enlarged to show some detail of cell size and arrangement. F-H. Growing point of poisoned embryo. Note the broadening of leaf primordia and of growing point. Compare with B and C. I-J. Enlarged detail of tips of poisoned seedlings, showing enlarged cells and hypertrophied primordia. K-L. Cross sections of poisoned seedlings showing enlarged cells and irregular outlines of hypertrophied tissues. M. Growing point and greatly hypertrophied leaf primordia of poisoned seedling. N-O.

cells, tissues and organs in proportion to the severity of the gross external symptoms. The first indications of histological abnormality in the plumule were found to occur in the older leaf primordia, in the form of isolated areas of enlarged cells (Fig. 2, I). At this stage the apical meristem and the younger leaf primordia are normal as to cell size and organization. As a rule, hypertrophy begins near the end of a primordium and progresses downward, the effect becoming evident in surface cells rather than in the cells of the interior of an organ (Fig. 2, J). Hypertrophic activity eventually spreads to the youngest leaf primordia and, finally, to the apical meristem. The formation of new cells and new leaf primordia ceases, the existing cells continuing their excessive, irregular enlargement. As the result of this activity, elongation of the axis and organs ceases, but these structures become greatly thickened. The outlines of the axis and leaf primordia exhibit irregular lobes and crenations (Fig. 2, F, I-M) suggestive of wound callus or certain types of gall. This resemblance is superficial, for the former types of wound hypertrophy are characterized by hyperplasia, whereas the hypertrophy of corn seedlings presents the more uncommon condition of hypertrophy without cell multiplication. The absence of hyperplasia is true only in the sense that cell multiplication does not accompany nuclear division.

The cells of the hypertrophied tissues of corn seedlings were found to be multinucleate. The number of nuclei in a cell varies from one to more than ten. Where many nuclei are present in a cell, the number is difficult to determine with certainty because the nuclei are clustered. In general, the larger cells contain more nuclei than the smaller cells. The nuclei of hypertrophied tissues, and in fact within a multinucleate cell, vary greatly in size and chromatin content. The diameters of small and large nuclei may be in a ratio as large as 1:20. The large nuclei frequently exhibit lobes (Fig. 2, N, O). Late prophase figures (Fig. 1, D) show that the several nuclei in a cell undergo independent, though usually simultaneous, mitosis.

The "giant nuclei" are clearly polyploid. The late metaphase figure (Fig. 1, B) has at least 100 chromosomes, or perhaps fragments. The chromosome group in figure 1, C, contains well over 200 chromosomes. Anaphase figures show an irregular multipolar separation of chromosomes, resulting in the formation of some small nuclei containing a few chromosomes, and the reincorporation of most of the numerous chromosomes in one or more polyploid giant nuclei. Cell wall formation appears to be inhibited, although spindle fibers and fragmentary wall plates are discernible. The formation of aneuploid and polyploid nuclei, and of multinucleate cells, is clearly the result of abnormal and incomplete mitosis.

The mechanism of these abnormal mitoses resembles various features of

the numerous cases cited by Kostoff and Kendall<sup>2</sup> and by Politzer,<sup>3</sup> but unlike many known cases, the abnormality in poisoned corn seedlings is most active in the meristems and primordia. Varying degrees of hypertrophy are observable in poisoned seedlings and there is a possibility of recovery, or perhaps survival to maturity. If recovery, and the persistence of the induced polyploidy can be demonstrated by experiments now in progress, this method of inducing polyploidy may be well worth the attention of plant breeders.

#### SUMMARY

An anatomical study was made of the hypertrophy produced in seedlings of corn by treatment with ethyl mercury phosphate.

The leaf primordia become much thickened, and develop irregular cre-nations and lobes.

In the leaf primordia and apical meristem of the plumule, cell division is inhibited; the existing cells undergo very great enlargement.

Cells of the hypertrophied organs become multinucleate, containing nuclei that range in size from minute "micronuclei" to very large "giant nuclei." The latter are polyploid.

The multinucleate condition and the formation of micronuclei and giant nuclei are the result of abnormal, incomplete mitosis.

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<sup>2</sup> Kostoff, D., and J. Kendall. Studies on plant tumors and polyploidy produced by bacteria and other agents. *Arch. Mikrobiol.* 4: 487-508. 1933.

<sup>3</sup> Politzer, Georg. *Pathologie der Mitose*. 238 pp. Gebrüder Borntraeger, Berlin. 1934.

# SCLEROTINIA ROT OF IRISH POTATOES

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## INTRODUCTION

A sclerotium disease of Irish potatoes caused by *Sclerotinia sclerotiorum* (Lib.) Massee was reported first from Ireland by Pethybridge in 1910 (4, p. 241-256). Lachaine (3) reported that it was present in New Brunswick, Canada, in 1922, but his photographs show that the sclerotia resemble those of *S. minor* Jagger more closely than those of *S. sclerotiorum*. Lachaine (3) also recorded the occurrence of the disease in England, Scotland, and Australia, and Brien (2) found it in New Zealand. In the United States, the disease has been reported from New York and Florida (1), Washington,<sup>1</sup> and Montana (6). *Sclerotinia sclerotiorum* also attacks many other plants (2, 6) and is one of the most important fungi causing decay of vegetables in transit (5).

## THE DISEASE

Aside from Pethybridge's report that the disease was important in Ireland (4), no reports indicate that sclerotinia rot has caused any particular loss of potatoes in any locality, except Hastings, Florida, where it did some damage in 1933 and was very prevalent in 1934. During that year it killed 15 to 70 per cent of the plants in several fields, totaling 120 acres, and caused a 25 per cent reduction in yield in the most severely affected fields. There was, however, very little loss in this area in 1935.

The hosts of *Sclerotinia sclerotiorum*, as listed by Young (6), include many wild and cultivated plants that are widely distributed throughout the United States. At Hastings, Florida, plants found attacked by the fungus in and near potato fields are as follows: cabbage, *Brassica oleraceae* var. *capitata* L.; tomato, *Lycopersicon lycopersicon* (L.) Karst.; ragweed, *Ambrosia elatior* L.; sow thistle, *Sonchus oleraceus* L.; calendula, *Calendula officinalis* L.; fireweed, *Erechtites hieracifolia* L. Raf.; and water cress, *Radicula obtusa* Nutt. Green. The last 3 plants have not been previously reported as hosts of the fungus.

The fungus attacks the tops of potato plants, but does not infect the tubers. The main stem may be attacked at any point, but it usually is invaded at the soil line. The first sign of the disease on the stem is the appearance of a water-soaked lesion, followed by the development of white mycelium of the fungus on the surface. The epidermis in the infected area dies and turns brown as the lesions become 2 to 6 inches long. All except the

<sup>1</sup> U. S. Dept. Agr. Bur. Plant Indus. Repr. Sup. 86: 47. 1935. [Mimeographed].



lignified tissues of the invaded parts are destroyed, and the interior of the stem becomes packed with mycelium and black sclerotia. The plant wilts when the water-conducting tissues are destroyed and finally collapses and dies. Any of the branches and leaves of the plant may become infected and the white mycelium is evident on the parts affected. The sclerotia, which are formed on the surface of the leaves and branches and inside the main stem and large branches, are of irregular shape and are  $\frac{1}{8}$  to 1 inch long. The symptoms of the disease are illustrated in figure 1.

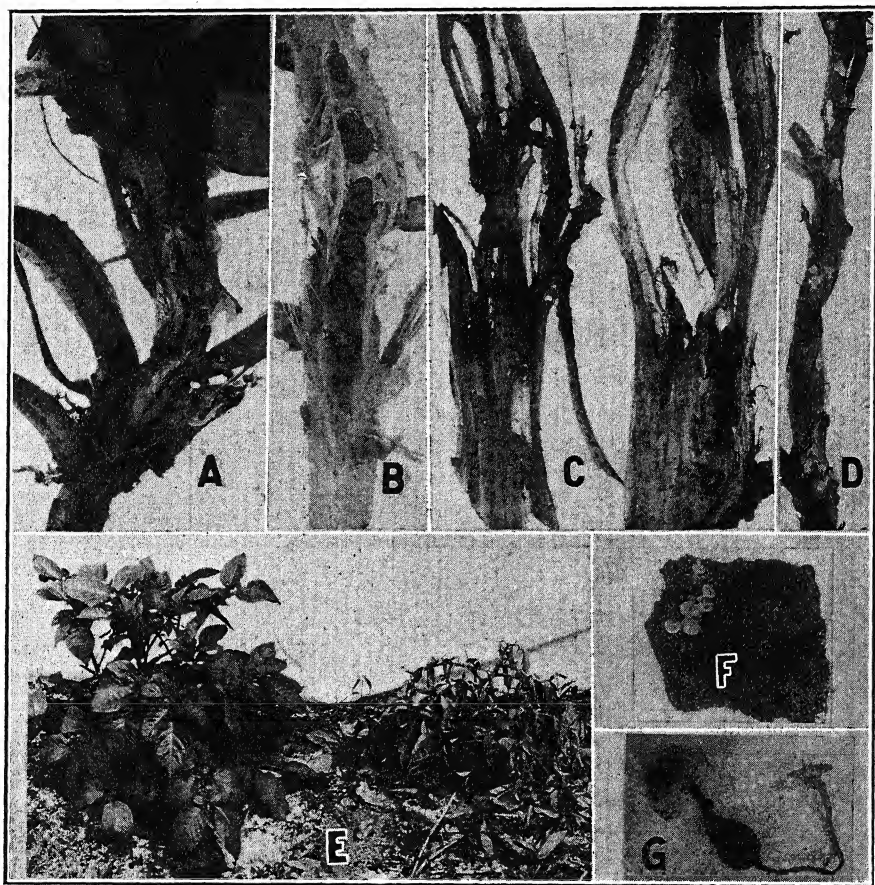


FIG. 1. Sclerotinia rot of potato. A. Lesion on lower part of stem. B. Stem spread open to show sclerotia. C. Shredded stem with parenchymatous tissue destroyed by the fungus. D. The fungus growing on surface of diseased branch. E. Healthy plant (left) and a plant wilted by sclerotinia rot of main stem. F. Six apothecia produced by one sclerotium. G. One sclerotium with two apothecia formed under field conditions.

Cool, rainy weather in March and April has been most favorable for the development of the disease in the Hastings, Fla., section. Such conditions prevailed in 1933 and 1934, when the disease was severe. In 1935, when the latter part of the growing season was hot and dry, it caused very little loss. Fogs and heavy dews also have been favorable. Furthermore, the greatest damage has occurred in potatoes on low ground that had remained wet for a prolonged period. The disease becomes most severe near the end of the growing period, when the dense foliage of the plants provides shade that favors growth of the fungus on the lower branches and leaves.

#### CAUSAL ORGANISM

*Appearance of the Apothecia:* In 1935, apothecia of *Sclerotinia sclerotiorum* were first observed in the field on February 8, 11 days prior to the appearance of the disease on the potato plants. The apothecia grew under the shade of the plants and in other locations in and between the rows. From 1 to 6 apothecia were seen arising from a single sclerotium (Fig. 1, F and G). The apothecial cups were well-expanded and measured from 1.5 to 10.0 mm. in diameter; the stipes varied from 2.5 to 24.0 mm. in length.

*Infection:* Infection is caused by ascospores, which germinate and invade potato stems and leaves with which they come in contact, and by mycelium, which comes in contact with healthy parts of the plant, as it grows on the soil and diseased potato stems and leaves. The disease is most severe on the parts of the plant nearest the ground, but some plants are affected only in their tops, indicating that infection in such cases was directly due to the ascospores, a fact proved by placing a potato plant, which had been grown in a pail, under a bell jar and by suspending apothecia, obtained from the field, inside the jar. The disease appeared first in the top of the plant nearest the apothecia that discharged the ascospores. The plant finally was killed and sclerotia were produced on the leaves and branches and inside the stem.

*Cultural Characteristics and Pathogenicity of Sclerotinia sclerotiorum, S. minor, and S. intermedia.*<sup>2</sup> When *S. sclerotiorum*, *S. intermedia* Ramsey and *S. minor* Jagger were grown in Petri plates on hard potato-dextrose agar, *S. sclerotiorum* formed mycelium more abundantly and produced larger, but fewer, sclerotia than either of the other species; *S. minor* produced the scantiest growth of mycelium and the smallest but most abundant sclerotia (Fig. 2).

Mycelial cultures of each of the 3 species, grown on potato-dextrose agar, were used to inoculate potato plants, kept in a moist chamber, by placing the mycelium of each species in contact with the nonwounded leaves and branches of each of 20 plants. *S. sclerotiorum* infected all of the 20 plants

<sup>2</sup> The culture of *Sclerotinia intermedia* was obtained from Dr. G. B. Ramsey, University of Chicago, Chicago, Ill., and *S. minor* from Dr. H. H. Whetzel, Cornell University, Ithaca, New York.

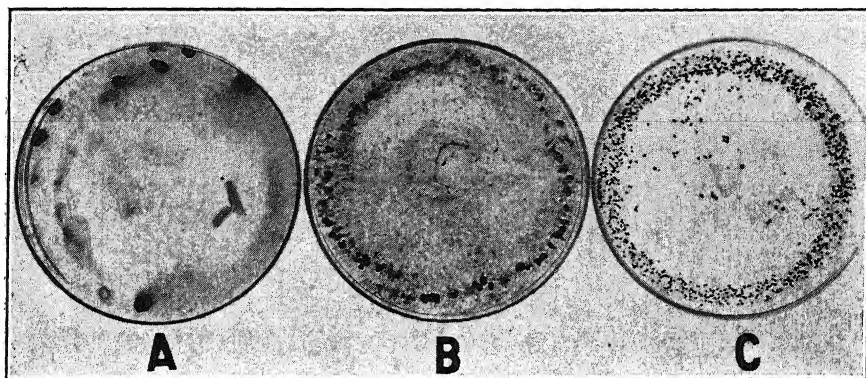


FIG. 2. Petri plate cultures of three species of *Sclerotinia*, grown for 30 days on potato-dextrose agar. A. *S. sclerotiorum*. B. *S. intermedia*. C. *S. minor*.

inoculated, and *S. minor* infected 11; symptoms of the diseases produced by both species could be distinguished only by comparing the size of the sclerotia of the 2 organisms from the diseased parts of the plants. *S. intermedia* did not infect the plants and formed no sclerotia.

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## PHYTOPATHOLOGICAL NOTES

*The Possibility of Ribes Infection by Aeciospores of Cronartium ribicola at Temperatures above 19° C.*—The minimum, optimum, and maximum temperatures for the germination of aeciospores of *Cronartium ribicola* Fischer, in water, have been reported as 5°, 12°, and 19° C., respectively.<sup>1</sup> While conducting classes in culture methods during the past few years, the writer noted that fresh aeciospores of this fungus germinated readily in water at room temperature (approximately 21° C.). The writer also obtained infection of *Ribes rotundifolium* by inoculating a potted bush with aeciospores of *C. ribicola* and placing it in a damp chamber, where the temperature never dropped below 21° C. Since the blister rust fungus is gradually advancing into regions where its ability to cause infection of *Ribes* species at temperatures above 19° C. may prove to be of importance in blister rust control, it was deemed worthwhile to determine the highest temperatures permitting aeciospore germination.

On May 9, 1936, living bark with mature aecia of *Cronartium ribicola* was removed from each of 5 infected trees of *Pinus strobus*, growing in the Pack Demonstration Forest at Warrensburg, New York. The pieces of bark were placed in separate containers and, the following day, were brought to Syracuse, New York, where they were left, uncovered, in a laboratory for 48 hours. Examination of the spores at the end of that period disclosed no pregermination. On May 12, suspensions of aeciospores in distilled water were made in separate culture tubes from each of the 5 cankers. By means of a wire loop, drops of each spore suspension were drawn across the surface of solid water agar contained in Petri dishes. The free water was absorbed by the agar, and thus the aeciospores were brought into direct contact with the surface of the agar medium, which supplied sufficient moisture to permit spore germination. The Petri dishes were then covered and separated into 10 groups of 5 cultures each, such that each group contained 1 culture of spores originating from each of the 5 cankers. The cultures were placed in thermostatically controlled incubators with the exception of 1 set of cultures, which was placed in a container out-of-doors. The temperatures at which the cultures were incubated included those temperatures determined by Doran to be the optimum and maximum for aeciospore germination. The incubation temperatures were recorded on thermometers, except in the case of the out-door cultures, for which a standard thermograph was employed.

The cultures were examined at the end of 12 and 18 hours. The percentage of germination was approximately the same for the spores from each of the 5 cankers; hence the results were not recorded separately for the

<sup>1</sup> Doran, W. L. The minimum, optimum, and maximum temperatures of spore germination in some Uredinales. *Phytopath.* 9: 391-402. 1919.

TABLE 1.—*The germination of aeciospores of Cronartium ribicola at different temperatures*

Temperature °C.	At the end of 12 hours			At the end of 18 hours				
	Spores counted	Germination	Maximum length of germ tube	Spores counted	Germination	Maximum length of germ tube	Average length of germ tube	Infection index <sup>c</sup>
	<i>No.</i>	<i>Per cent</i>	$\mu$	<i>No.</i>	<i>Per cent</i>	$\mu$	$\mu$	
Out-door temperature <sup>a</sup> .....	500	83	732	500	83	1098	915	8
12 <sup>b</sup> .....	500	79	475	500	83	732	658	5
18-19 ...	500	71	732	500	73	732	549	4
20-21 ...	1000	72	534	1000	74	732	549	4
22-23 ...	500	70	366	500	74	732	549	4
24-25 ...	500	75	732	500	78	732	549	4
26-27 ...	500	40	732	500	66	732	439	3
28 .....	500	28	534	500	31	732	439	1
29 .....	500	0	.....	500	0	.....	.....	.....
30 .....	500	0	.....	500	0	.....	.....	.....

<sup>a</sup> During the first 12 hours the temperature dropped steadily from 21° C. to 12° C. During the remaining 8 hours it ranged between 11° C. and 12° C.

<sup>b</sup> Optimum temperature as recorded by Doran.

<sup>c</sup> Per cent germination  $\times$  average length of germ tubes. The results are divided by 10,000 and given in round numbers.

individual cankers. The resulting data are shown in table 1. Of particular significance are the facts that the percentage of germination decreased less rapidly in relation to increased temperatures than was found to be the case by Doran; and the maximum temperature for the germination of aeciospores was 28° C. instead of 19° C. Although aeciospores germinated at 28° C., the majority of the young germ tubes soon became vacuolate and at the end of 18 hours most of them appeared empty and apparently dead. The length of the germ tubes was measured but, because they were somewhat curved and twisted, it was impossible to obtain their exact length. However, the lengths recorded are approximately correct. After 18 hours, the aeciospores incubated at out-of-door temperatures averaged somewhat longer germ tubes than those spores incubated within a narrow temperature range. No explanation is advanced for this phenomenon. In nature, however, spores are normally subjected to fluctuating temperatures during germination.

The results obtained are significant in determining the relative possibility of *Ribes* infection from aeciospores of *Cronartium ribicola* at various tem-

peratures. When considering the possibility of infection, the length of the germ tubes at the end of a definite period is as important as the ability of the spores to germinate. If the germ tubes are not sufficiently long to extend from the spores into the mesophyll tissue, infection will not occur. Measurements taken on leaves and sections of leaves from 4 species of *Ribes* showed that half the average distance between the stomata plus the distance from the stomata to the mesophyll tissue was less than 400  $\mu$ . Thus a relative index to the possibility of infection at different temperatures at the end of 18 hours was obtained by multiplying the percentage of germination by the average length of the germ tubes. The infection index (Table 1), calculated from the data obtained in this study, indicates that infection may occur at temperatures as high as 28° C. within 18 hours, but that it is more apt to occur at the lowest temperatures used in this experiment.—RAY R. HIRT, Department of Forest Botany and Pathology, New York State College of Forestry, Syracuse, N. Y.

*A Bacterial Wilt and Soft Rot of the Potato in Maine.*—Within recent years the writer's attention has been called to a soft rot and wilt of potatoes in Aroostook County, Maine. The disease was first noted by the writer in 1932 in a field where 20 per cent of the tubers were decayed by a soft rot. A former seed-potato inspector, however, has stated that it was present in some fields of Maine several years prior to 1932. Since 1932 it has appeared annually in certain localities in Aroostook County.

Up to the present the disease has been most commonly observed in the vicinity of Fort Fairfield. It also has been found farther north toward New Sweden, and specimens have been received from Presque Isle. B. Baribeau has reported a very similar disease from the Province of Quebec, Canada.<sup>1</sup>

This soft rot of the potato apparently is not yet generally distributed in Aroostook County. The losses in a few individual fields, however, have been large. One small field in 1934 showed 30 per cent of the plants affected and fully one-third of the crop decayed, either in the field or in the bin. In Maine it generally is noted in late summer, when the tubers are well formed. The disease was present to a less extent on several other farms in the same vicinity. In 1935 it was again present on one of these farms, causing a 20 per cent loss in portions of a field and also some damage in other fields in the same vicinity. The writer has observed it mostly on more or less poorly drained soil. Some growers, however, maintain that it frequently occurs on the higher and well-drained soils.

It has been observed most commonly on the Green Mountain variety, but was found also on the Irish Cobbler and Katahdin varieties.

<sup>1</sup> Baribeau, B. Geographical distribution of bacterial blight of potatoes in Quebec. Ann. Rep. Quebec Soc. Prot. Plants 27 (1934/35): 80-83. Illus. (map). 1935.



The first aboveground evidence of the disease is a wilting of leaves and individual stalks (Fig. 1). The leaves on the wilted stems become chlorotic

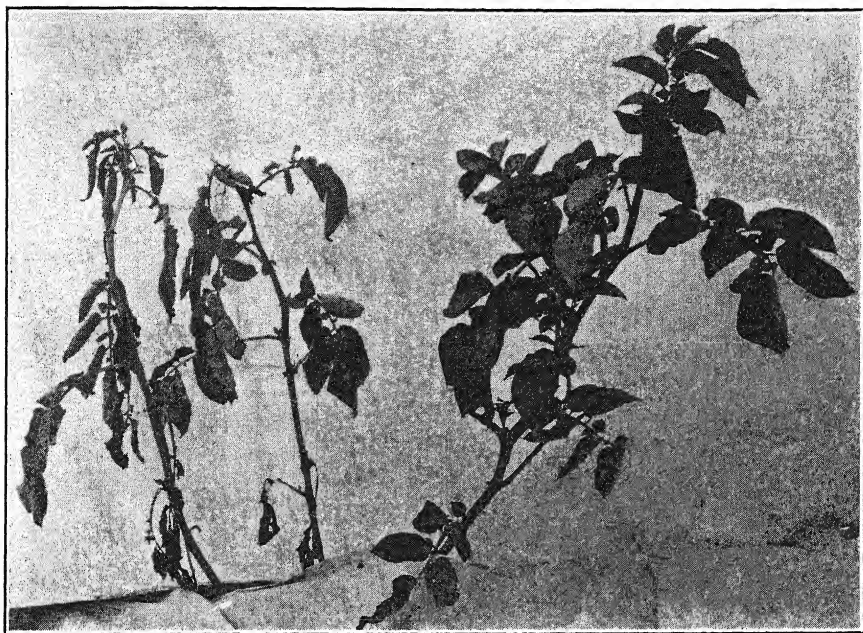


FIG. 1. Green Mountain potato plant showing typical wilt symptoms in the field. Note healthy stalk on the right.

and gradually die. They sometimes show marginal necrosis, and the vascular bundles of the stems appear more or less discolored. On digging, it is noted that the tubers originating from the wilted stems are decayed. The decay appears to begin in the region of the vascular system and thence extends into the pith, causing a white or cream-color rot (Fig. 2). Often the entire center of the tuber disintegrates, leaving the mere shell. These hollow tubers frequently are found in a bin of potatoes from an affected field.

The tubers from affected plants, if harvested before the rot sets in, often show a characteristic cracking (Fig. 2).

The disease is perpetuated through the seed tubers. In 1935 apparently healthy tubers were harvested from diseased plants. Most of these tubers, although apparently disease-free, decayed in storage. Thirty-six plants, however, were raised from the seed stock that survived, 5 of which showed typical wilt symptoms. Most of the 36 plants developed tubers with characteristic cracking when harvested in the fall. Some growers insist that

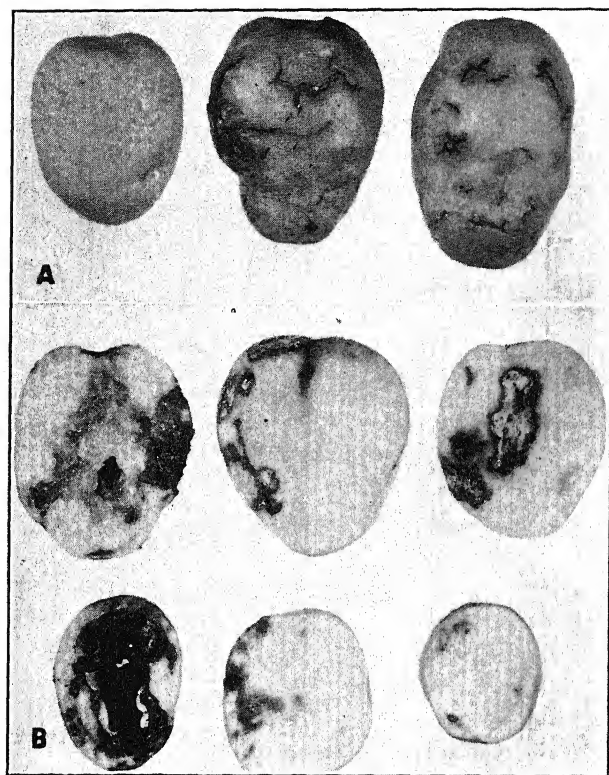


FIG. 2. A. Two tubers showing typical cracking. Healthy tuber on the left. B. Halved tubers showing different stages of vascular discoloration and decay.

their potatoes acquired the disease through seed stock introduced from other farms.

The writer has attempted to identify the organism responsible for this disease. A bacterium having the salient physiological and morphological characteristics of *Bacillus carotovorus* L. R. Jones has been most commonly isolated. This organism, isolated from wilt-diseased plants, is capable of causing a white soft rot of potato tubers, but in no case has it produced the symptoms of the disease in question, on inoculation, nor has it been found capable of causing blackleg when introduced into potato stems. Another organism very similar to the soft rot has been isolated that is characterized as a rapid gas former on the various sugar media. Several other bacteria (not related to the soft-rot bacteria) have been secured from the tissue of diseased potatoes, but in no case have inoculations with these organisms produced the symptoms of this disease.

Growers are of the opinion that use of new seed stocks has eliminated the disease from their respective farms.—REINER BONDE, Maine Agricultural Experiment Station, Orono, Maine.



*Comparative Studies on Cultures of Phytomonas lactucae-scariolae, n. sp. and Phytomonas pruni.*—A leaf-spot disease of wild lettuce, *Lactuca scariola*, observed by the writers on June 25, 1929, at Urbana, Illinois, appeared to be of bacterial origin from microscopic examinations. Sectioned and stained material demonstrated the location, as well as the presence, of bacteria in the infected tissue. Since no reference to this disease was found in the literature, and, since the organism in culture appeared similar to *Phytomonas pruni* (E. F. Smith) Bergey *et al.*, 1930, a comparative study of morphological and biochemical properties of the two organisms was carried out under identical cultural conditions following the Manual of Methods for Pure Culture Study of Bacteria prepared by the Society of American Bacteriologists. These results and a description of the disease on wild lettuce are here reported.

The lesions on wild lettuce, on first appearance, are circular and water-soaked, but they develop rapidly into decidedly angular spots. The mature lesions are from 2 to 4 mm. in diameter and the tissues are shrunken and light brown. A distinguishing character of the bacterial spots from other lesions is the glistening oily appearance given the under, and sometimes upper, surface by the exudate produced from bacterial growth in the tissue. Free-hand sections of the spots showed bacteria oozing from the margin of diseased and healthy tissues, when examined microscopically in water mounts.

The organism on wild lettuce was repeatedly isolated from lesions by the dilution-plate method, and produced typical symptoms 5 days after atomizing 6-day-old cultures to healthy wild-lettuce plants kept in moist chambers 36 hours prior to and 24 hours after inoculation. Reisolations yielded typical colonies of the pathogen. Pathogenicity of *Phytomonas pruni* has been determined previously from pure cultures isolated from diseased tissue. Inoculations with cultures of *P. lactucae-scariolae* to peach foliage and green shoots and with culture of *P. pruni* to wild lettuce were negative in all cases, showing different pathogenicity for each organism. The morphological and biochemical properties and cultural features of the two organisms were indistinguishable in all measurements. Although the bacteria differed only in their pathogenicity the evidence seems inadequate to consider these as related strains of bacteria. For this reason, for the custom of naming bacteria according to the host infected, and to avoid confusion with *Bacillus lactucae* Voglino<sup>1</sup> the name *Phytomonas lactucae-scariolae, n. sp.* is proposed for the organism pathogenic to wild lettuce. The results are given in the technical descriptions.

*Phytomonas lactucae-scariolae, n. sp.* is a short rod, 1.0 to 1.5  $\mu$  long

<sup>1</sup> Elliott, Charlotte. Manual of bacterial plant pathogens, 1930, Baltimore, The Williams Wilkins Company.

and 0.5 to 1.0  $\mu$  wide; motile by 1 and 2 polar flagella; occurs singly, in pairs, and in short chains, but mostly in pairs; is capsulate, but forms no endospores or granules; is Gram-negative and not acid-fast; forms round, entire, finely granular, amber-yellow colonies on dextrose agar; clouds bouillon, and forms a yellowish rim, but no pellicle; has slight diastatic activity; liquefies gelatin slowly after 10 days; reduces nitrates, but does not produce hydrogen sulphide; produces very slight acid reaction and causes peptonization in litmus milk, with reduction of litmus and slight coagulation; does not produce gas from xylose, rhamnose, glucose, mannose, galactose, fructose, maltose, sucrose, rhaminose, raffinose, dextrin, inulin, glycerol, manitol, sorbitol, ducitol, or salicin; is aerobic; optimum, minimum, and maximum temperatures and H-ion concentrations for growth are 25, 7, and 35° C. and pH 7.5, pH 4.8, and pH 11.0, respectively; causes dark irregular spots on leaves of wild lettuce, *Lactuca scariola*, but is non-pathogenic to cultivated lettuce, *Lactuca sativa*.

*Phytomonas pruni* (E. F. Smith) Bergey *et al.*, 1930, having the same morphological and biochemical characteristics as *P. lactucae-scariolae*, causes irregular lesions on leaves, green shoots, and fruits of peach, *Amygdalus persica*, but is non-pathogenic to wild lettuce. These properties are similar to those listed by Elliott (2) for the organism.—H. H. THORNBERRY<sup>2</sup> AND H. W. ANDERSON, Department of Horticulture, University of Illinois, Urbana, Illinois.

*Control of Peach Leaf Curl by Autumn Applications of Various Fungicides.*—While making tests of various fungicides for the control of peach blight (*Coryneum beijerinckii* Oud.) it became necessary to determine whether the spray applied for this disease (an application after the leaves are off, usually between November 15 and December 15), also would prevent leaf curl [*Taphrina deformans* (Fckl.) Tul]. Although the time most frequently set for applying the leaf curl spray is the early spring before the buds begin to open, there is evidence<sup>1,2,3</sup> that both Bordeaux mixture and lime-sulphur applied in the autumn adequately controls the disease. Recently, however, Fitzpatrick,<sup>4</sup> working in Ontario, tested the efficiency of lime-sulphur applied at different times in autumn and spring. He found that autumn sprays gave poorer control of leaf curl than those applied in early spring. If the autumn spray should fail to prevent leaf curl in California,

<sup>2</sup> Department of Agronomy, University of Kentucky, Lexington, Ky.

<sup>1</sup> Smith, R. E. California peach blight. California Agr. Expt. Sta. Bull. 191. 1907.

<sup>2</sup> Reddick, D. and L. A. Toan. Fall spraying for peach leaf curl. New York (Cornell) Agr. Expt. Sta. Circ. 31. 1915.

<sup>3</sup> Berkeley, G. H. Fall spraying for peach leaf curl. Canad. Hort. 47: 245. 1924.

<sup>4</sup> Fitzpatrick, R. E. The life history and parasitism of *Taphrina deformans*. Sci. Agr. 14: 305-326. 1934.

a second application in early spring would be necessary, since fungicides applied much later than December 15 fail, in many years, to control peach blight.

With these points in mind, plots of from 16 to 20 trees were sprayed at different times and with different materials during the autumn and spring of 1935-36. Results taken on 2 different dates in the spring appear in table 1.

TABLE 1.—*Results of spraying for the control of leaf curl on Elberta peach trees, 1935-36*

Treatment <sup>a</sup>	Leaves infected on March 13	Leaves infected on April 7
	<i>per cent</i>	<i>per cent</i>
Nonsprayed, Plot 1 .....	42	46
“ Plot 2 .....	33	43
“ Plot 3 .....	29	36
“ Plot 4 .....	30	45
Bordeaux mixture, 2-5-50, Oct. 24 .....	0.7	0.6
“ “ 5-5-50, Oct. 24 .....	0.2	0.2
“ “ 5-5-50, Nov. 29 .....	0.1	0.1
“ “ 5-5-50, Jan. 29 .....	0.4	0.1
“ “ 5-5-50 + oil, Nov. 29 ...	0.2	0.3
Lime-sulphur, 4-50, Nov. 29 .....	0.5	1.0
Basic copper sulphate, 3-50, Nov. 29 .....	0.1	0.3
Copper ammonium silicate, 3-50, Nov. 29	2.2	13.0

<sup>a</sup> All Bordeaux mixture was made with unslaked lime.

Bordeaux mixture + oil = a dormant oil emulsion added to Bordeaux mixture at the rate of 4 gallons per 100 gallons of spray.

Lime-sulphur = a standard brand of the liquid material.

Basic copper sulphate = Basicop, a material containing 52 per cent metallic copper, with a spreader, recommended by the manufacturer, added at the rate of  $\frac{1}{2}$  pint per 100 gallons of spray.

Copper ammonium silicate = Coposil, a material containing 22 per cent metallic copper, with one gallon of a dormant oil emulsion added to each 100 gallons of spray.

The first spring infection undoubtedly was initiated during a prolonged rain, which lasted until the tips of the first two or three leaves were exposed. A second wave of infection, appearing between March 13 and April 7, probably was initiated during a series of rains between March 26 and April 4.

Bordeaux mixture, 5-5-50, gave excellent control, whether applied on October 18, November 29, or January 26 (about three weeks before buds began to swell). Bordeaux, 2-5-50, Bordeaux-oil, and Basicop applied in autumn also gave good control. Coposil, on the other hand, did not prevent the disease so efficiently as did the other materials. Although no spring application of lime-sulphur was made, control could not have been significantly better than that afforded by the autumn application.

Fitzpatrick's studies of the fungus afford an explanation of the difference between the results of his tests and those of the present tests. In the first place, Fitzpatrick sprayed single branches, while in the present test the entire tree was sprayed. Fitzpatrick maintained that sprout conidia, produced by budding of the ascospores, are spread throughout the tree by rain. If, therefore, all of the sprout conidia in the tree are not killed by the fungicide, they are likely to be carried to sprayed branches after the spray is washed away by rain. The reason his autumn application failed to control the disease was because the conidia from nonsprayed branches spread to the sprayed ones after the spray coating weathered away. This spring application gave good control because the spray coating, retaining its effectiveness until the leaves appeared, prevented the conidia from reaching and infecting the leaves. In the present tests, on the other hand, the entire tree having been sprayed, there remained in the tree no large amount of viable inoculum to spread to the leaves when they appeared. There was, of course, the possibility of lateral drifts of conidia from the other trees, but no evidence of this was seen in autumn-sprayed trees adjacent to nonsprayed plots.—E. E. WILSON, Division of Plant Pathology, Branch of the College of Agriculture, Davis, California.

*Blooming of Potatoes as Influenced by Pyrethrum Dust.*—In connection with studies on the stimulation of potato plants by Bordeaux mixture it was noted in 1935 that pyrethrum dust (a mixture of 25 pounds of pyrethrum<sup>1</sup> and 75 of Celite<sup>2</sup>) used as an insecticide had a decided effect on the foliage development of the plants. Further tests were conducted in 1936 to determine the merits of this material to induce increased foliage weight, as well as to continue the study of the stimulating effect of Bordeaux mixture on plants, where the insects were largely controlled by pyrethrum. The experiments have not yet been completed (September 1, 1936), but a striking phenomenon, that of increased blooming, has occurred in the pyrethrum-treated plants. Under the rather poor growing conditions in the 1935 field, no blossoming occurred on any of the plots.

The total number of blossom clusters, blooming and not blooming, was recorded in 1936 on a 100-plant basis (Table 1). Two noteworthy tendencies may be noted. First, pyrethrum applied in addition to Bordeaux mixture practically doubled the number of blossom clusters, and an even greater increase took place on the plants that were not sprayed with Bordeaux mixture, that is, if pyrethrum alone was used. Secondly, more than half the total blossom clusters opened their flowers when pyrethrum dust

<sup>1</sup> A pyrethrum dust, "Powco A," procured from John Powell and Co., Inc.

<sup>2</sup> A relatively inert powder consisting chiefly of silica and other inert ingredients procured from the Johns Manville Co.

TABLE 1.—Number of blossom clusters on 100 plants under different dust and spray treatments

	Bordeaux mix. alone			Unsprayed	Bordeaux mix. + pyrethrum dust			Pyrethrum dust alone
	1a	2a	3a		1a	2a	3a	
No. of blossom clusters not blooming .....	143	162	108	95	116	121	114	137
No. of blossom clusters blooming .....	16	20	26	28	141	156	141	162
Total No. of blossom clusters	159	182	134	123	257	277	255	299

\* Different spray schedules.

was applied. When Bordeaux mixture alone was used only a small proportion of the flowers opened. A slightly higher number of flowers blooming was found in the unsprayed than in the Bordeaux sprayed plants. This has been commonly noted during a number of years in these experiments.

The nature of these phenomena is not yet understood. It is believed that something other than insect control is involved. Whether pyrethrum dust acts as a stimulant to the growth of the plant, remains to be proved. Experimental evidence concerning foliage (and tuber) weights of plants treated with pyrethrum in addition to copper suggests this latter possibility (unpublished data).—E. O. MADER AND E. C. UDEY, Department of Plant Pathology, Cornell University.

*Physiologic Races of Snapdragon Rust.*<sup>1</sup>—The heavy natural rust infection occurring on rust-resistant snapdragons<sup>2,3,4</sup> in certain coastal regions of California in 1936 indicated that a hitherto-nonrecorded race of the rust (*Puccinia antirrhini* Diet. and Holw.) may have appeared. To test this possibility rust-susceptible and rust-resistant plants were inoculated with rust from rust-resistant plants grown in several places in California, and with rust from rust-susceptible plants grown in Berkeley, where rust-resistant plants remained rust-free during the 1936 season.

Because of the unavailability of rust-free, rust-resistant, and rust-susceptible plants, and because of the convenience and reliability of the dish-culture method,<sup>5</sup> excised leaves were used for making the tests. Two rust-

<sup>1</sup> The writer wishes to acknowledge the cooperation of Dr. D. R. Porter and Mr. C. O. Blodgett in securing some of the cultures of rust and snapdragons used in this study.

<sup>2</sup> Emsweller, S. L. and Jones, H. A. The inheritance of resistance to rust in the snapdragon. *Hilgardia* (California Agr. Expt. Sta.) 8: 197-211. 1934.

<sup>3</sup> Mains, E. B. Studies in rust resistance. *Jour. Heredity* 17: 313-325. 1926.

<sup>4</sup> Mains, E. B. Rust resistance in *Antirrhinum*. *Phytopath.* 25: 977-991. 1935.

<sup>5</sup> Yarwood, C. E. The comparative behavior of four clover-leaf parasites on excised leaves. *Phytopath.* 24: 797-806. 1934.

TABLE 1.—*Differentiation of physiologic forms of snapdragon rust on excised leaves on 5 per cent sucrose solution in Petri dishes*

Host plant number	Previous history of plants in regard to rust resistance	Inoculated with physiologic race 1 of snapdragon rust		Inoculated with physiologic race 2 of snapdragon rust	
		Number of leaves inoculated	Total number of rust pustules	Number of leaves inoculated	Total number of rust pustules
17 .....	rust-susceptible	11	209	11	70
18 .....	rust-susceptible	10	198	11	96
1 .....	rust-resistant prior to 1936	18	0	18	389
2 .....	rust-resistant prior to 1936	13	0	12	307

susceptible but rust-free greenhouse plants and several rust-free and rust-resistant plants growing beside heavily infected rust-susceptible plants, outdoors at Berkeley, were used as host material. Vigorous leaves were distributed in Petri dishes with their dorsal surfaces upward, 2 Petri dishes of leaves from each test plant. One Petri dish of leaves from each plant was dusted with urediospores from rust-susceptible plants grown in Berkeley, and the other with urediospores from rust-resistant plants obtained in another locality. The dusted leaves were atomized with water. Five per cent sucrose solution was placed in each dish, and the inoculated leaves floating on the sugar solution were incubated in diffuse light at room tem-

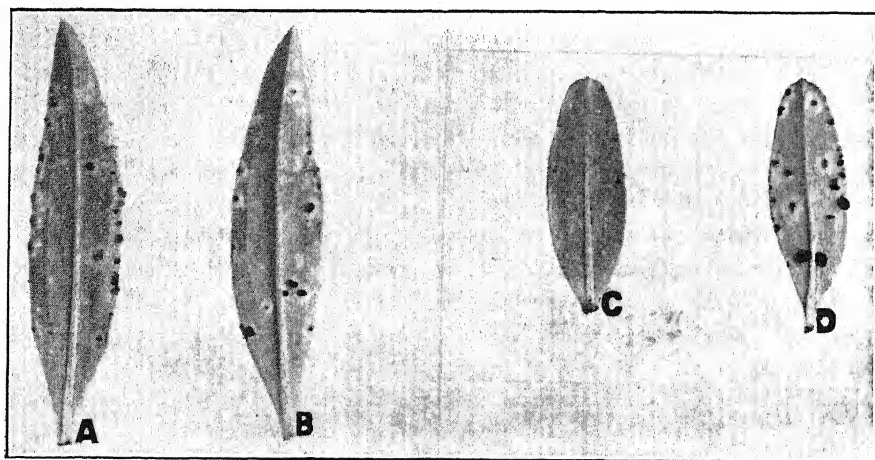


FIG. 1. Differentiation of physiologic races of snapdragon rust on excised leaves on 5 per cent sucrose solution in Petri dishes. Photographed 13 days after inoculation. A. Rust-susceptible plant inoculated with race 1. B. Rust-susceptible plant inoculated with race 2. C. Rust-resistant plant inoculated with race 1. D. Rust-resistant plant inoculated with race 2.

perature (19–23° C.). Rust pustules were evident in about 7 days; many had broken through the epidermis in 10 days. Representative results in the first test, 13 days after inoculation, are represented in table 1 and figure 1.

The leaves of rust-susceptible plants became heavily infected with all rust collections, but the leaves of rust-resistant plants became infected only with the rust from rust-resistant plants. Rust-resistant snapdragons were immune from rust collected at Berkeley in 5 tests, and to rust occurring at Davis and Sacramento in 1 test. The susceptibility of rust-resistant snapdragons to rust occurring 5 other localities in coastal California has been demonstrated in 5 tests. These results clearly indicate that there are at least 2 races of *Puccinia antirrhini*. The rust to which the resistant plants are resistant will be termed physiologic race 1, and that infecting the rust-resistant selections will be termed physiologic race 2. Races 1 and 2 may each comprise several races of rust, as the writer did not work with pure cultures, but no evidence of further races was observed. Of 11 tested plants of different genetic lines of rust-resistant snapdragons, none have proved to be resistant to race 2.—CECIL E. YARWOOD, Division of Plant Pathology, University of California, Berkeley, California.

*A Method of Inoculating Seed Barley with Black Loose Smut for Use in Studies on Physiologic Races.*—In previously reported studies<sup>1</sup> on the black loose smut of barley (*Ustilago nigra* Tapke), the writer obtained high percentages of smutted heads as a result of blackening the seed with dry smut spores. Although effective and easy to apply, this method presents an inherent difficulty when used in physiologic race studies involving many different smut collections that must be prevented from mixing. The dry spores are so volitant that it is difficult or impossible to keep them confined, even by the most careful handling, in the preparation of inoculum by removing the spores from the smutted heads and in applying the smut dust to the seed. Furthermore, if the dusted seed is placed in envelopes and the latter are squeezed in handling, puffs of smut emerge from the corner vents of the envelopes and a further spread of the smut occurs.

To avoid this difficulty the writer has devised a wet method of preparing the inoculum. The latter is then applied to the seed by the spore-suspension method,<sup>2</sup> recently described for inoculating seed barley with covered smut. The method as used in recent studies of physiologic races of *Ustilago nigra* is as follows: Four loose smut heads are immersed in 750 cc. of

<sup>1</sup> Tapke, V. F. A study of the cause of variability in response of barley loose smut to control through seed treatment with surface disinfectants. Jour. Agr. Res. [U. S.] 51: 491–508. 1935.

<sup>2</sup> Tapke, V. F. An effective and easily applied method of inoculating seed barley with covered smut. Phytopath. 25: 1038–1039. 1935.

water in a 1-liter Erlenmeyer flask. By vigorously shaking the flask the spores are loosened from the heads and suspended in the water. The suspension is then poured into another vessel through a fine screen to remove the remnants of the smutted heads and any other extraneous matter. This spore suspension is then used to inoculate thoroughly dry seed, previously treated by the modified hot-water method for the prevention of loose or covered smuts. The fluid is poured over small lots of seed in shell vials until it rises about  $\frac{3}{4}$  inch above the seed. The seed is vigorously shaken in this suspension for  $\frac{1}{2}$  minute, then allowed to soak 15 minutes. The suspension then is decanted and the vials are inverted on clean pieces of blotting paper to absorb all free water. Next, the vials of moistened inoculated seed are packed, uncorked, in a tightly covered tin box floored with a wet blotter to maintain high humidity, and then incubated for 24 hours at 18–20° C. Lastly, the seed is transferred to small envelopes, crimped to remain wide open, where it is left 2 or 3 days, or until thoroughly dry. It is then ready to sow. Through the cooperation of the North Carolina Agricultural Experiment Station and the Division of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, a field planting of 25 varieties of winter barley, each inoculated by this method with 10 different collections of *U. nigra*, was made at Statesville, N. C., in the fall of 1935. Despite the severe winter of 1935–36, high percentages of smutted heads, reaching a maximum of 83 per cent were obtained in the spring of 1936. It is, therefore, evident that the method is highly effective. Moreover, it has the desirable feature of practically eliminating the hazard of mixing the smut collections, so troublesome in the dust method.—V. F. TAPKE, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.



## BOOK REVIEW

Boysen, Jensen P. *Growth Hormones in Plants*. Authorized English translation of *Die Wuchsstofftheorie* . . . Translated and revised by George S. Avery, Jr., and Paul R. Burkholder, with the collaboration of Harriet B. Creighton and Beatrice A. Scheer. 1st Ed. 268 pp. McGraw-Hill Book Company, New York and London. 1936. \$3.50.

Bibliographies: pp. 221-253.

While this book is of primary importance to those interested in plant physiology, phytopathologists will find it interesting despite the fact that bacteria and fungi have not been included in the scope of the work. However, there is a fairly comprehensive list of references dealing with bios, folliculin, and other sex hormones, and those affecting the growth of fungi. The historical review is cleverly treated by means of sketches and explanatory notes. Throughout the book the distribution, detection, and quantitative determination of growth substances are demonstrated by the curvature responses of the coleoptile of *Avena*. These substances have been detected and in some cases have been isolated in chemically pure form from many sources, such as human urine, corn oil, malt, yeast, *Aspergillus*, *Rhizopus*, etc. In addition, numerous synthetic compounds have been shown to possess similar growth-inducing properties in various degrees of intensity. "Hormones" have been demonstrated in many green plants belonging to widely separated taxonomic groups, ranging from *Avena* to *Sequoia*, and from *Lupinus* to orchids. Numerous fungi, bacteria, and at least one alga, *Valonia*, have been shown to possess growth-inducing properties. The formation of such substances in fungi seems to be influenced by glucose peptone, glucose-ammonium tartrate, tryptophane, tyrosin, etc. While it is comparatively simple to demonstrate the presence of growth substances in plants, their transportation in the tissues presents certain difficulties. The translocation seems polar, but is abolished by anaesthesia. It is shown that no growth of shoots of higher plants can take place without the "hormones," yet the elongation of the roots is inhibited by these substances; this suggests a selective toxicity. It is possible that these growth substances are not used as "building stones" but as activators.

It seems that this growth substance is formed in expanding buds and leaves, whence it moves into other parts of the plant, exercising some control over growing parts, and under some conditions promoting callus tissues, tumors, and roots. Nastic responses, prolongation of growth period and prevention of petiole abscissions also have been shown to be influenced by auxins. Phototropic curvatures are influenced by the unequal displacement of growth substances, whereby the illuminated sides of the plant receive less

auxins than the shaded sides. Similarly, geotropic, traumatic, and thigmatic curvatures have been shown to be influenced by auxins.

The title of the book is perhaps a bit unfortunate, as the term hormone belongs to animal physiology and constitutes the product of specialized glands. Furthermore, at least some of the substances, treated by the author as hormones, may not be true auxins but toxic substances that irritate the plant into an excessive production of auxins. Looking upon the phenomenon of excessive callus, root, and tumor formation under the action of these alleged hormones, the plant pathologist can see a pathological condition induced by the presence of substances inimical to the plant. Witches'-broom, hairy root, tumors, galls, etc., are all too familiar examples of the action of bacteria and fungi upon their host plants. It is more logical to assume that such abnormal growths are the result of excessive production and concentration of auxins in an attempt to overcome the invader than to presume that the pathogen furnishes growth substances to the host plant. If such be the case, then we still have to isolate in chemically pure form the true growth substances found in green plants.—LEON H. LEONIAN, West Virginia University, Morgantown, West Virginia.

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## ABSTRACTS OF PAPERS ACCEPTED FOR PRESENTATION AT THE TWENTY-EIGHTH ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY, ATLANTIC CITY, NEW JERSEY, DECEMBER 28 TO 31, 1936<sup>1</sup>

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*Development and Experiences in the Use of Apparatus for Pouring and Washing Agar Plates.* W. E. AHRENS.

A machine designed to pour agar plates automatically and deliver them hardened, ready for use in a few minutes, has been developed. Sterile plates are inserted in a hopper from which they pass on an endless belt beneath a cylinder that delivers a measured quantity of medium to the plate. Lids are removed and replaced by a synchronized vacuum system. The poured plates then pass to a cooling tank from which they emerge hardened, ready for use in 5 minutes at the rate of 14 plates a minute.

Another machine of the endless-belt type has been devised to clean and wash poured plates. Lids and bottoms are separated by an operator and fed into the machine in separate slides. The medium is removed by a jet of steam striking the inverted plate. Wax pencil markings are erased by a sheepskin belt saturated with xylene. Plates now pass through a tank of boiling soapsuds and then they are rinsed, top and bottom, by streams of fresh water sprayed on them from a centrifugal pump. After rinsing, the plates emerge at the rate of 14 a minute and are reassembled by a second operator.

*Distribution of Spores of Wilt-inducing Fungi Throughout the Vascular System of the Elm by the Sap Stream.* W. M. BANFIELD.

Spore suspensions of fungi inducing elm wilts, including *Ceratostomella ulmi*, were injected into the bases or tops of 4- to 8-inch elms from early spring to late autumn. Three hours to 3 weeks after injection the limits of distribution of the spores were determined by identification of the fungi in cultures made from centrifuged sap displaced from various levels in the tree or from the subsequently discolored wood. Injection of spores was achieved by cementing a funnel-shape pan around the stem, filling the pan with spore

suspension, severing in contact with and under the suspension all actively conducting vessels. Sap displacements were made by cementing metal collars around the upper ends of stem sectors, filling the collars with sterile water and collecting the liquid that dripped from the stem bases.

The maximum distribution of the fungi in trees after 3 days in April was 2 inches above and 2 feet below the points of injection. Spores were recovered 30 feet above points of injection after 3 hours in June and 24 feet below points of injection after 2 days in October. *C. ulmi* spores were removed from the sap stream of naturally diseased trees in May, July and September.

*Possible Relationship of Stanley's Crystalline Tobacco-Mosaic-Virus Material to Intracellular Inclusions Present in Virus-infected Cells.* HELEN PURDY BEALE.

Cytoplasmic inclusions, characteristically present in cells affected with certain strains of tobacco-mosaic virus, are the vacuolate, plasma-like, "x-bodies," and crystalline material, often striated, that occurs in plates, frequently hexagonal, in side-view oblong.

Strips of epidermis from the midribs of leaves, affected with Johnson's tobacco virus 1 or 6, were mounted in water and observed under high magnification. Acid, approximately pH 1.3, was run under the cover slip, and, as the acid penetrated the cells, the oblong crystalline masses developed cross striations, finally breaking up into needles that floated out free in the cells. The crystalline plates formed needles with their long axes at right angles to the basal plane of the plate. Excess acid dissolved the needles, leaving the x-bodies and host nucleus intact.

Using 3 different acids, intracellular precipitation of needle-shape crystals was obtained with 6 different hosts. Needle-shape crystals were not formed in healthy tobacco cells nor in those affected with the viruses of tobacco ring spot or potato x.

In gross appearance, the intracellular-needle crystals were indistinguishable from those formed upon acidification of virus extract, purified according to Stanley's method.

*The Dutch Elm Disease in Europe.* R. KENT BEATTIE.

The elm is widely distributed in Great Britain. Five species and 2 varieties are recognized. They hybridize freely. The elm disease was first found in 1927. It was already widely distributed and eradication efforts were soon abandoned. It is now within 30 miles of the Scottish border. No *Cephalosporium* or *Verticillium* is known in England. The cause of a dieback present in the North of England and Scotland is as yet unknown.

In The Netherlands, elms formerly bordered most of the roads. The disease was first observed at Tilburg in 1919 and was seen nowhere else. It is now widely scattered. Much creditable research has been performed by the Dutch investigators. For 5 years a systematic clean-up has been under way not to eradicate but to delay spread and permit economical utilization.

The elm disease has been in Belgium certainly for 18 years. No efforts to control it are being made.

In France the disease was first noted at Chalons-sur-Marne in 1919. No effort to control it is being made. It was seen between Calais and Paris, around Versailles, at Vichy in central and Bergerac in southwest France.

Burl elms, such as brought the disease to America were seen widely distributed in France and England. Uniform evenly grown burls are not common.

*A Comparison of Linted and Acid-delinted Cotton Seed.* J. G. BROWN.

The sulphuric acid cotton-seed delinting treatment is a valuable control measure for angular leaf-spot. In addition, it is much easier to sow delinted seed. It has been found that linted and delinted seed do not respond the same when planted at different depths in

wet, heavy soils, and that to take full advantage of the many superior qualities of delinted seed, proper planting is important. These differences in the behavior of linted and delinted seed have been associated with seed air-carrying capacity; to measure which a simple apparatus has been specially devised.

*Symptoms and Terminology in Some Physiogenic Apple Diseases.* A. B. BURRELL.

"Cork," as commonly applied to apple symptoms, denotes rather large, definitely delimited masses of dead cells within the fruit; drouth spot, extensive areas of superficial necrosis. (1) Correlation in occurrence, (2) parallel response to cultural treatments, and (3) grafting experiments, indicate that cork, drouth spot, a form of dieback and a form of rosette, as occurring in the Lake Champlain Valley, compose a symptom-complex and are not to be regarded as separate diseases. The same symptom-complex evidently occurs in New Zealand, British Columbia, and elsewhere. A name is needed for the disease as a whole, but its choice should await fuller knowledge of the rôle of boron. (See Burrell and Miller abstract.) The variety of apple largely governs whether it takes on the cork type or the drouth spot type of symptom or a combination. The relation to this disease of a late-summer diffused, interior browning of apple flesh, largely controllable by irrigation, is not quite settled. Drouth spot often is mistaken for frost injury or spray injury. Heat injury, sometimes classed with drouth spot, is distinct. The rosette here discussed, is distinct from the zinc-responsive rosette of western U. S., and, of course, from peach rosette.

*Boric Acid Treatment of a Physiogenic Apple Disease.* A. B. BURRELL and H. J. MILLER.

The symptom complex of this disease includes drouth spot, cork, rosette, and dieback. (See Burrell abstract.) Directly after petal fall, in crowns of 12 young McIntosh and Fameuse trees, a single hole, usually 7/16" diameter, was bored 2" diagonally downward, 0.75 to 2.0 grams U. S. P.  $H_3BO_3$  crystals introduced, and hole sealed. When treated, these trees had 1335 rosette twigs of which 92.4 per cent resumed normal growth within 6 weeks. In 7 trees receiving  $ZnSO_4$  flakes only 2.0 per cent of 866 rosette twigs recovered. In 6 nontreated checks, with 769 rosette twigs, only 1.4 per cent recovered. On August 10, incipient dieback showed on 1.1 twigs per  $H_3BO_3$  tree, 60.4 twigs per  $ZnSO_4$  tree, and 65.5 twigs per check. On August 10 an estimated 2.2 per cent of all twigs showed abnormal second growth on  $H_3BO_3$  trees, 22.3 per cent on  $ZnSO_4$  trees, and 20.2 per cent on check trees. Dry  $H_3BO_3$  caused appreciable injury around the hole, but no foliage injury.  $ZnSO_4$  caused both types of injury.  $H_3BO_3$  introduction in mature trees gave promising, but inconclusive, results as to fruit symptoms, chiefly because of inadequate yields. Injection of 5 per cent  $H_3BO_3$  solution was highly injurious; 1 per cent usually was not. Soil application of  $H_3BO_3$  crystals for 4 years was noninjurious, but disease symptoms were insufficient to permit conclusions.

*Cultural Characters of *Thyrostroma compactum*, from Chinese Elm.* J. C. CARTER.

Although the writer previously reported inability to grow *Thyrostroma compactum*, in culture, subsequent attempts have been more successful. Conidia from a canker developed after November 1, 1935, were collected and planted on potato-dextrose agar May 8, 1936. On May 9 they had germinated, but hyphal growth proceeded very slowly, the linear extension being 40-172  $\mu$  in 19 hours and 46-452  $\mu$  in 65 hours. Conidia planted May 30 on 5 types of agar: potato-dextrose, corn-meal, malt-extract, malt-extract with *Ulmus pumila* twig decoction, and 3 per cent plain agar with twig decoction, germinated on all. Growth was most rapid on corn-meal and plain twig-decoction agar and slowest on potato-dextrose. Maximum colony diameter, about 3 mm., was attained in 12 days. Conidia remained viable 22 days in the ice box (about 50° F.) but were dead at the end of 3 months. Ger-

minating conidia transplanted to sterilized *U. pumila* twigs or to agar slants did not produce visible growth. The two end cells germinated most frequently, but, not uncommonly, muriform cells germinated at the same time. Germ tubes from basal cells usually branched during the first 24 hours, those from tip cells usually after 48 hours and frequently not until after reaching a length of 200  $\mu$  or more. In no case were cell contents observed to pass entirely into germ tubes during germination.

*The Limitations of Plant Virus Serology.* K. STARR CHESTER.

The blood test for virus identification and classification has been applied to some 60 viruses and virus strains. About half of these give group-specific precipitin tests, the others fail to react serologically. There is a positive correlation between specific seric activity of the virus juices and their ability to retain infectivity when subjected to dilution, heating, and ageing. In general, the viruses which show seric activity are capable of mechanical transmissibility at expressed-juice dilution up to 1:1000, they resist heating up to about 55° C., and they withstand ageing for 3-4 days or more. This correlation between virus properties and seric reaction suggests that it is the viruses themselves that are responsible for the blood reactions, although the possibility that the reactions are due to by-products of virus activity is not excluded. Failure of virus juices to give specific blood reactions may be due to low concentration, antigenic inactivity, or instability of the virus antigens. In exceptional cases it might be due to the presence of an undetected masked virus strain in the supposedly healthy plant juices used for absorbing the sera. Further progress in virus concentration and stabilization may extend the list of viruses amenable to seric techniques.

*Spraying as a Method of Control for Mildew (Peronospora tabacina) and Wildfire (Bacterium tabacum) in Tobacco Plant Beds.* E. E. CLAYTON.

The experiments were conducted over the period 1932-1936, inclusive, with the object of developing a spray procedure that would give effective disease control and would not increase transplanting hazards. Many materials and methods of application have been tested. Best results were obtained by a combination of early weekly Bordeaux sprays, followed later by semi-weekly applications of a copper oxide-cottonseed oil spray. This program has been subjected to extensive field tests for 2 years. The results show that field stands from sprayed plants were either equal or superior to those obtained from unsprayed plants; that mildew was reduced to such a degree that it caused no damage, and that almost perfect control of wildfire was obtained. These results were obtained without the aid of soil sterilization, seed treatment, or other sanitation measures.

*Germination of Conidia of Peronospora effusa from Spinach.* HAROLD T. COOK.

Various factors affecting germination of conidia of *Peronospora effusa* from spinach were studied with spore suspensions on glass slides. The conidia were found to germinate over a temperature range of from slightly above 0° to 30° C. The optimum temperature was between 10° and 15° C. Newly formed conidia did not germinate so well as those a day old. Conidia germinated after 5 days' exposure on detached infected leaves in the laboratory, but remained viable for only 3 days when exposed on dry glass. In some tests the conidia germinated better in dew than in distilled water or lake water. Dew from several plants appeared to be as favorable for germination as that from spinach.

*Sclerotinia sclerotiorum on Pyrethrum.* HAROLD T. COOK.

*Sclerotinia sclerotiorum* was the cause of a very destructive disease in an experimental planting of *Chrysanthemum (Pyrethrum) cinerariaefolium* at Norfolk, Virginia, in the spring of 1930. Counts showed that the disease increased from 9 per cent infected plants on April 28 to 12 per cent dead and 60 per cent infected plants on May 28. Symp-



toms of the disease are wilting of leaves followed by collapse of young stems. Frequently only part of the plant is affected at first, and the collapse of the stems causes a bald or nest-like spot in the plant. Later, the entire plant becomes infected and dies. The fungus mycelium is present in the crown of the plant in the early stages of the disease and soon gives rise to sclerotia, both on the surface and within the stems. Apothecia were found arising from sclerotia buried in the ground and from those inside the stems.

*Cucumber Mosaic in Puerto Rico.* MELVILLE T. COOK.

During the winter of 1935-36 we had a severe outbreak of mosaic, amounting in many cases to as much as 100 per cent infection. This is the first severe outbreak observed in Puerto Rico in 13 years. The writer's studies failed to show the source of the infection. His studies showed the same morphological characters of the host as reported by him for the other virus diseases.

*The Witches'-Broom of Tabebuia pallida Caused by a Virus.* MELVILLE T. COOK.

This disease has been attributed to a fungus. Tissue inoculations have demonstrated that it is caused by a virus. It has not been transmitted by seeds.

*Savoy, a Virus Disease of Beet Transmitted by Piesma cinerea.* G. H. COONS, J. E. KOTILA, and DEWEY STEWART.

Affected plants (sugar beet, garden beet) show dwarfed, down-curved, savoyed leaves, most pronounced effects being found on innermost leaves. Primary symptoms are veinlet clearing followed by thickening of veinlets, giving the dorsal leaf surface a netted appearance. Roots of affected plants show, in late stages, phloem necrosis and flesh discoloration, simulating curly-top effects. From initial partial leaf- and root-involvement, the disease becomes general. Affected roots test low in sucrose. The virus has been transmitted by adults of *Piesma cinerea*, viruliferous and non-viruliferous individuals being found. The virus overwinters in affected plants and in the vector. Incubation period in sugar beet is 3 to 4 weeks. Attempts at transfer by means of juice, aphids (*Mysus persicae*, *Aphis rumicis*) and leaf hoppers (including *Eutettix tenellus*) have been unsuccessful. The disease has general distribution in Michigan, Ohio, Minnesota, Nebraska, South Dakota, Colorado, and Wyoming, incidence ranging from trace to 5 per cent. The disease described for Indiana by Arthur and Golden (1892) and "leaf curl" noted in Michigan (1901) may represent early records. "Savoy" differs distinctly from curly top, and from the German *Kräusellkrankheit* of beets transmitted by *Piesma quadrata*. The disease may be expected to remain a relatively minor factor affecting crop production.

*Bunch Disease of Pecans.* J. R. COLE.

Bunch disease of pecans, which, in the spring of 1932, was definitely determined to be new to that species, was first observed in the Red River Valley of Louisiana, near Shreveport. The disease is thought to have first attacked the wild native pecan, *Hicoria pecan*, and the water hickory, *Hicoria aquatica*, both species being indigenous to the alluvial river-bottom soils and spreading gradually to the susceptible improved varieties of *H. pecan*. The bunch disease is known to be present in Louisiana, Mississippi, Oklahoma, and Texas. Characteristic and distinguishing symptoms of this disease are the brooming of branches and shoots, early foliation of diseased branches in the spring, chlorotic, thin, broad, wavy, and flexible leaves, and, in later stages, dying back of the branches. Bunch has certain symptoms similar to rosette and little-leaf of pecans; two important diseases of peaches, phony peach and yellow peach, and witches'-broom of black locust. The disease was successfully transmitted by grafting diseased Schley scions onto healthy Schley stocks. The Schley and Mahan varieties are very susceptible, while the Stuart is highly resistant to the disease.

*Wetwood.* BOWEN S. CRANDALL, CARL HARTLEY, and R. W. DAVIDSON.

At the meeting of two years ago there was reported a bacterial vascular disease in the Salicaceae, characterized by a water-soaked condition of the central wood. It now appears that this wetwood is of frequent occurrence, in most cases without perceptible effect on the health of the tree. It has been found commonly in 9 species or distinct forms of *Populus*, 3 of *Salix*, and 5 of *Ulmus*; in single species of *Abies*, *Tsuga*, *Prunus*, *Morus*, and *Prosopis*, in young *Platanus* and in cuttings of *Elaeagnus*; and rarely in the inner sapwood region of 3 species of *Quercus*. Fermentation odors are noticeable, particularly in elm; the wetwood where tested by direct application of an indicator has shown pH values higher than live sapwood and much higher than generally shown by true heartwood. Bacteria have been obtained in culture from wetwood in representatives of all the above phanerogamic genera except *Prosopis*. Pathological phenomena which seem to be associated in some cases are the dying of Lombardy poplar, *Salix caprea*, *Platanus* sp., *Quercus borealis*, and cuttings of *Elaeagnus*; slime flux in several species, and subsequent decay due to secondary fungus infection. Preliminary inoculations with bacteria from *Platanus* sp. produced wilt in *Platanus* cuttings.

*The Interaction of Two Apple-rotting Fungi.* H. R. X. D'AETH.

Bramleys Seedling apples were inoculated with *Sclerotinia fructigena* and *Penicillium expansum* separately, and with a mixture of the two together. The percentage weight of rotted tissue determined for each inoculation was converted to give the mean penetration of the fungus in centimeters. Thus, the rate of rotting could be followed accurately through all stages. The development of the rot was divisible into (1) an "early" phase, in which rotting proceeded very slowly, and (2) a "late" phase, characterized by a uniform high rate of rotting. During the "late" phase the rates of rotting by the mixture and by *S. fructigena* were closely similar, and exceeded that caused by *P. expansum* alone. Isolations from mixed rots showed that the growth of *P. expansum* was the same in tissue previously rotted by *S. fructigena* as in normal apple tissue. With *P. expansum* the "early" phase lasted about as long as with *S. fructigena*; the "early" phase of the mixture was shorter than that of *S. fructigena* at 10° C., and longer at 15° C., indicating a complex reversible interaction.

*Reduction of Bordeaux Mixture Injury by the Use of Amendments.* ROBERT H. DAINES and WILLIAM H. MARTIN.

Tests have been conducted during the past season in which amendments have been added to a 1-3-50 Bordeaux mixture to render it less injurious to apple foliage and fruit (Golden Delicious variety). Three types of amendments were used: (1) metals above copper in the electro-motive series were used to reduce soluble mobile copper to metallic copper; (2) zinc sulphate was used to reduce the solubility of the copper in the Bordeaux films; and (3) a wettable sulphur was used to increase the loss of copper caused by weathering.

Copper analyses of foliage were made at regular intervals to determine the influence of the amendments on the adherence of the copper in the Bordeaux films. The metals referred to in Group 1, reduced foliage and fruit injury from a trace to as high as 75 per cent without increasing the loss of copper from the foliage by weathering. The representatives of Groups 2 and 3 reduced the adhesiveness of the copper in the Bordeaux films to a certain extent, and proved only slightly effective in reducing copper injury.

*Nitrogen Supply of Sugar Beets in Sand Cultures in Relation to Extent of Injury by Southern Sclerotium Rot.* A. E. DAVEY.

The work here reported attempts to test for presence or absence of resistance to attack of *Sclerotium rolfsii* in sugar beets as a result of differential nitrogen supply

to beets grown in mineral nutrient solution in sand culture. The nitrogen supply was varied by depriving the culture solution of nitrogen (ammonium nitrate) after different periods of development of the beets in the several lots. After heavy inoculation the time required for the disease to cause permanent wilting was in general shorter for the low nitrogen groups. Examination of the lifted roots showed that neither the depth of necrotic tissue at point of inoculation nor the superficial necrotic areas of the roots were greater in low than in high nitrogen groups. The difference in time required for wilting was apparently due to the longer time required to involve the absorptive roots in the larger beets in the high nitrogen group. It is possible that the extreme conditions of inoculation may have obscured real differences in susceptibility operating under field conditions.

*Cultural Identification as a Necessary Supplement to Tree Decay Studies.* ROSS W. DAVIDSON, W. A. CAMPBELL, and DOROTHY J. BLAISDELL.

Cultural identification studies conducted during the past 3 years in connection with stand-improvement operations of the Civilian Conservation Corps have revealed unusual and little-known fungi to be the cause of considerable decay in trees. The more important ones isolated include *Stereum gausapatum*, *Polyporus compactus*, *Poria andersonii*, and *Corticium lividum* from oaks, *Polyporus glomeratus* from maple and *Stereum murrayi* from birch. The following characters based on an extensive reference collection of authentic cultures are used in the identification of organisms isolated from decayed trees: (1) Growth rate at different temperatures; (2) color and texture of mycelial mat; (3) fruiting in culture; (4) odor; (5) oxidase reaction; (6) microscopic appearance.

The identity of the organisms responsible for decay in living trees is basic to the application of intelligent methods of control. Identification by means other than cultural comparison is subject to doubt because many wood-inhabiting organisms cause similar types of decay, sporophore production is rare in the majority of heart-rotting organisms, and several different organisms may be present in a single tree.

*The Cercospora Leaf Spot of Rose Caused by Mycosphaerella rosicola.* B. H. DAVIS.

In the study of cercospora leaf spot of rose, conducted at Ithaca, New York, an ascigerous stage was found in overwintered leaves. Single ascospore isolations and inoculations proved this to be the perfect stage of *Cercospora rosicola* Pass. This stage has characteristics of the genus *Mycosphaerella*, but unlike any described species. The combination *Mycosphaerella rosicola* (Pass.) comb. nov. is proposed. Only species and varieties of *Rosa* are known to be affected. The range and symptoms of the disease are given. The pathogenicity is proved by inoculation experiments.

Although 9 names have been applied to species of *Cercospora* on rose, a comparative study shows only two distinct species among them. These are *Cercospora rosae* (Fckl.) v. Höhn., which is unknown in the United States, and *Cercospora rosicola*, which is world-wide. Among specimens deposited in the Cornell University herbarium, a specimen of a *Cercospora*, collected at Savannah, Georgia, was found that is distinct from the two mentioned above. Additional material was obtained from Florida. This species, known only in the southern States, is given the name *Cercospora pudarii* nov. spec.

*Some Disease Developments in Forest Nurseries.* W. C. DAVIS, D. H. LATHAM and GEO. Y. YOUNG.

Expansion in forest and erosion-control planting has resulted in special attention to nursery diseases, with active cooperation between the Bureau of Plant Industry, the Forest Service, and the Civilian Conservation Corps. In 1936 the heaviest losses resulted from drouth and heat. A permanent needle droop of red pine, similar to an undiagnosed condition prevalent in the Lake States in 1935, has attended drouth injury in Maryland

and been produced experimentally in the greenhouse by withholding water. The droop effect apparently results from collapse of recently grown tissue at the base of the needle. Deficiency troubles, relieved in different cases by the application of iron, nitrogen, or phosphorus, were observed; purple discoloration of all but the bases of the terminal needles characterized the early stages of the phosphorus deficiency in pine. Use of chicken manure on red-pine transplants was directly correlated with an epidemic of root rot associated with *Fusarium moniliforme*. A *Phytophthora* resembling *P. parasitica* fruited luxuriantly on killing lesions on black-locust stems up to  $\frac{1}{4}$  inch in diameter.

*Seed Treatment in Relation to Sand Culture of Seedlings.* A. A. DUNLAP.

Seeds of a number of different flower and vegetable plants were treated according to the usual chemical and hot-water methods. These were planted, together with non-treated seed, in washed sand (plus nutrients) and in treated and nontreated soil. In sand, beneficial results were obtained from treatment of the seed only in certain exceptional cases. Injury to germination or to seedling growth sometimes resulted from treatment. Seed treatments for cabbage and Kochia have been found desirable. Seeds of most of the species investigated require no treatment under sand culture, to prevent damping off.

*Corticium Disease of Turf.* L. E. ERWIN.

*Corticium* disease was first found in Rhode Island in 1932. There seems to be little difference in susceptibility of colonial, creeping, and velvet bents to this disease. Temperature studies show that *Corticium fuciforme* has a temperature range between 8 and 30° C. The optimum temperature for maximum growth lies between 18 and 20° C. Experimental tests, conducted with a number of commercial products, showed that good control could be obtained with either inorganic or organic mercuries.

*Environmental Conditions Influencing the Development of Tomato Pockets or Puffs.*  
ARTHUR C. FOSTER.

Data collected during 4 years of intensive study, under greenhouse conditions, upon the growth of 8 crops of tomatoes under long and short day periods, with different approximate soil moistures, widely different fertilizer formulae, and different temperatures, indicate that tomato pockets may be caused by a number of widely varying environmental factors. It was found that any condition interfering with the normal fertilization and development of the ovules will result in fruit with empty locules or so-called "pockets." When tomato plants are grown continuously in a 60° F. mean-temperature house, with a minimum of 55°, all of the fruits are parthenocarpic with hollow, puffy locules, or typical pockets. Sudden marked changes in the available soil moisture at the time of fertilization of the ovules also leads to sterility and typical pockets. Either excessive soil-moisture or drought conditions during the fertilization of ovules interferes with the normal development of the placental area of the fruit and also results in parthenocarpic fruit. Very often a definite easily visible necrosis appears in the placental area, indicating death of these tissues. The same environmental conditions that cause blossom-end rot also may cause puffy tomatoes; in fact, puffiness often appeared as a forerunner of blossom-end rot in these studies.

*Environmental Factors Influencing the Development of Blossom-end Rot of Tomatoes.*  
ARTHUR C. FOSTER.

Over a period of 4 years, a detailed, intensive study has been made of such environmental factors as soil moisture, nutrition, and air temperature as may influence the development of blossom-end rot of tomatoes. With widely varying soil-moisture conditions, it was found that maximum soil moisture was more favorable to the development of the

disease than minimum amounts of soil moisture. In fact, plants grown continuously in low soil moisture appear to be resistant to blossom-end rot, due apparently to their hardened condition. Plants grown in optimum soil-moisture conditions will invariably develop the disease after exposure to drought. Fertilizers, especially the amount of nitrogen and phosphate, appear to have a very direct influence on the development of blossom-end rot. Increasing amounts of nitrogen appear to favor the development of the disease when other conditions are favorable. Increasing amounts of phosphate appear to have a very marked palliative effect in reducing the disease, even when soil moisture conditions favor its appearance. When other conditions are favorable, temperature does not influence the development of the disease, since it appears frequently at mean temperatures of 65°, 70° or 75° F. Furthermore, neither the water requirement nor the amount of water utilized by the plant has any relation whatever to the appearance of the disease. With increasing amounts of nitrogen fertilizer, the plants become more economical in the use of water, but appear to be more susceptible to the disease.

*Factors Affecting the Prevalence of the Spotted Wilt Virus.* M. W. GARDNER, C. M. TOMPKINS, and H. REX THOMAS.

Certain localities apparently function as endemic centers or foci of infection from which there may be considerable spread by thrips of the spotted-wilt virus in the spring and summer. These localities are characterized by mild winters, no summer rainfall, and the presence of living host plants throughout the year. Apparently, the thrips are active in these centers throughout the year, but the virus is least abundant just after the winter rains, possibly because of a reduction in the thrips population. Besides certain ornamentals and winter crops, certain common winter weeds, such as mallow and chickweed, may harbor the virus. There is no indication that the virus is harbored in the native vegetation of uncultivated lands, as is the curly-top virus. Occurrence of the disease in regions remote from foci of infection is often traceable to introduction of the virus with imported transplants. In localities where the virus is prevalent, a lower percentage of infection occurs in celery, celeriac, spinach, peas, endive, and chicory than in tomatoes, peppers and lettuce, and no infection has been observed in onions, rhubarb, beets, and sugar beets, chard, globe artichoke, carrots, parsley, beans (*Phaseolus*), crucifers, or cucurbits. Potatoes, though susceptible, also appear to escape infection.

*Evaluation of the Geneva Experiment on Scab Control.* W. O. GLOYER.

For 7 years lime-sulphur solution (1-40) has been applied to Ben Davis trees in a series of sequences in order to evaluate either a single or a combination of sprays. The effect of the scab fungus and the fungicide were studied in relation to yellow leaf, frog-eye, defoliation, blooming, dropping of fruit, yield, resistance, and related subjects. In the present paper the scab problem only will be considered. The data appear to justify some reconsideration of the concepts of scab control. Overwintering conidia were found to play a greater part in the initial infection than is generally believed. Dormant branches, forced and incubated in the greenhouse, showed conidia infection of the scale leaves. A heavy rain (one-half inch or more), in the delayed dormant stage, apparently favors spread of overwintering conidia. Scab was controlled by 3 applications when spraying operations began either with the delayed dormant, pre-blossom, or calyx spray. The fungicide in the cover sprays prevented autumn infection on the under side of the leaves.

*Breeding Disease-resistant Chestnut Trees.* ARTHUR HARMOUNT GRAVES.

In order to replace the now nearly extinct American chestnut (*Castanea dentata*) with a disease-resistant, tall-growing type, the Brooklyn Botanic Garden has been cross-

ing various species and hybrids of *Castanea* during the past 7 years. At the present date nearly 200 hybrids, consisting of various combinations, of which 20 are new to science, are growing in the plantations. The best of these, at the end of its 5th year of growth, is nearly 15 feet high. A method is described for measuring the degree of disease resistance of each individual.

*Control of Club Root of Crucifers.* C. M. HAENSELER.

Studies on the control of club root (*Plasmodiophora brassicae*) of crucifers, conducted annually since 1927, indicate that the disease may develop in soils showing a pH of 7.5 to 7.8 for composite samples. A total of 11,000 lbs. of hydrated lime per acre, applied over a 4-year period and giving a soil reaction of 7.5 to 7.6, gave only 73 per cent healthy plants.

Studies of micro-soil samples after 8 years of liming showed that a soil with a pH of 7.87 for composite samples contained small local areas ranging in pH from 5.73 to 8.45. This uneven distribution in soil acidity may account for the failure to obtain perfect control in soils showing high pH values for composite samples.

Calcium cyanamide, used alone, failed to give adequate club-root control on an acid soil but aided when used in conjunction with lime.

Metallic mercury mixed with fertilizer and applied 2 inches below and 2 to the side of the seed at rates equivalent to 8.3 and 16.6 lbs. Hg per acre of 24-inch rows, reduced club-root infection to 10.0 per cent and 5.1 per cent, respectively, compared with 17.0 per cent when fertilizer alone was used.

*Cercospora Leaf Spot of Calendula.* J. G. HARRAR.

A leaf spot of *Calendula* spp., first noted in 1933, increased in severity in Virginia during the next 2 years. Symptoms of the disease were characteristic of a *Cercospora* leaf spot and typical *Cercospora* spores were regularly isolated from leaf lesions. No published study of the disease has been found in the literature, but it has been observed and reported on at least 4 different occasions. The earliest report was that of Saccardo, in 1898, and at that time he named the parasite *Cercospora calendulae*. Experimentally, it was determined that the fungus attacks host plants when they are 4 or more weeks of age, gaining entrance through the stomata. The disease progresses rapidly, frequently resulting in total destruction of the host plant prior to flower production. Infection from air-borne and soil-borne inoculum has been demonstrated, but there is no evidence that the organism is seed-borne. Varietal tests with 17 varieties of *Calendula* gave no evidence of varietal resistance. Single-spore cultures of the fungus on several media produced abundant mycelial growth, but conidiospores were not formed. Control was obtained with sulphur dust, lime sulphur, Bordeaux, and copper oxide sprays.

*Movement of Intracellular Bodies Associated with Peach Yellows.* ALBERT HARTZELL.

The movement of intracellular bodies associated with peach yellows was recorded by means of cinephotomicrography. Scenes have been prepared showing these bodies moving in the cells of living peach-leaf-petiole, blossom, and root-hair tissues, as contrasted with healthy tissues in which such bodies are absent or rare. Intracellular bodies similar in appearance were found in the intestinal wall and salivary glands of living infected *Macropsis trimaculata*, the leafhopper vector of peach yellows. These also have been photographed. Similar bodies were not found in corresponding tissue of noninfected leafhoppers. Whether the intracellular bodies in the infected leafhoppers bear a primary relationship to those found in diseased plant tissue or are secondary in nature, was not determined. When infected tissue was crushed on slides and the intracellular bodies were released into the cell juice, those from infected peach-leaf-petiole tissue and from the cells of the intestinal wall of infected leafhoppers showed marked motility. Whether the

movement of the intracellular bodies observed is due to vital activity or to purely physical forces has not been determined.

*Histological Studies of Infection and Sporulation of Peronospora tabacina in Tobacco Seedlings.* R. G. HENDERSON.

On tobacco seedlings growing in the greenhouse, spores of *Peronospora tabacina* germinate on the surface of the leaves and penetrate the epidermis directly. The end of the germ tube comes in contact with the leaf surface and usually forms a slight enlargement that might be called an appressorium. A small hyphal strand is then pushed through the upper wall of the epidermal cell. Within the epidermal cell, an enlarged hypha develops that usually sends out haustoria into the surrounding cytoplasm and also into the adjoining cells. The enlarged hypha continues to grow, or may send out a smaller hypha, until it comes in contact with the inner cell wall where again it forms an enlargement and passes through the cell wall by means of a small hyphal strand into the intercellular spaces of the mesophyll. In a similar manner the germ tube may penetrate a cell of a leaf-hair and the hypha pass from cell to cell until it reaches the mesophyll.

Conidiophores arise from stomata on either surface of the leaf, but under average moisture conditions are confined to the lower surface. Conidiophores bearing conidia have been observed completely imbedded in the spongy parenchyma.

*Inheritance of Plant Characters and Resistance to Fire Blight in Pear.* E. M. HILDEBRAND and S. L. HSIONG.

It is generally recognized that the most promising method for combating fire blight in pears is the breeding of blight-resistant varieties. In an attempt to find a basis for pear improvement and disease resistance, the writers have examined the pear-breeding material at the New York Experiment Station at Geneva where reciprocal crosses had been made of a number of standard varieties. In examining the plant characters of the parental varieties and of the  $F_1$  hybrids it was found that the maternal parent usually exerted the most important influence on the offspring. This was evidenced by the manner in which certain plant characters as leaf size and serration, fruit quality and stem thickness were transmitted and suggests the need for careful study of reciprocal crosses. The transmission of certain plant characters in the more blight-resistant varieties, Kieffer and Seckel, to their offspring was found correlated with the transmission of blight resistance measured by the percentage of positive inoculations and the length of blight lesion produced. Further study on the inheritance of plant characters in pear is necessary to the determination of the more exact relationship to blight resistance.

*Hereditary Factors Affecting Tobacco-mosaic Disease in Solanaceous Plants.* FRANCIS O. HOLMES.

In 3 solanaceous genera, Capsicum, Browallia, and Nicotiana, recent hybridization experiments have given new information regarding genes associated with necrotic-type versus mottling-type response to infection with tobacco-mosaic disease. In *Capsicum frutescens* an allelomorph of 2 previously reported disease-response genes controls a partially dominant necrotic-type response characterized by imperfect localization of virus. In *Browallia speciosa* a single dominant gene restricts virus to the site of inoculation and allows recovery by abscission of leaves bearing necrotic primary lesions; in some individual plants there is evidence of a second dominant gene, indistinguishable in effect, but segregating independently with respect to the first. A dominant gene for necrotic-type response originally found in *Nicotiana rustica*, and recently reported to have been transferred to a strain of *N. paniculata* by repeated crosses with this species, with subsequent self-pollinations, has now been carried from the necrotic-type strain

of *N. paniculata* to plants of 3 successive generations of hybrids with *N. tabacum*. A similar gene has been carried from *N. glutinosa* to 3 generations of hybrids with *N. tabacum*.

*Effect of Copper Sprays on Ripening of Tomatoes.*<sup>1</sup> JAMES G. HORSFALL, R. O. MAGIE, and C. H. CUNNINGHAM.

The ripening of tomatoes sprayed with 4-4-50 Bordeaux mixture seems to be delayed. The cumulative yield curve lags. The current explanation for this delay is that diseases defoliate nonsprayed plants, letting in the sun, which accelerates ripening. In the South, where the season is long and diseases usually are serious, the detrimental effects of Bordeaux are counterbalanced by disease control. In the North, with short seasons, the injurious effects usually overshadow beneficial ones. Studies on the development of individual blossoms on test plants in greenhouse and field give the following information: (1) The "delay of ripening" occurs in absence of defoliation diseases; (2) The major factor seems to be that Bordeaux kills young blossoms and thus causes the yield curve to lag; (3) Stunting of young plants by Bordeaux may delay blossoming and reduce setting of blossoms; (4) Time interval between blossoming and ripening is unaffected by spraying, at least in absence of blight; (5) Cuprous oxide of equal copper content is less detrimental to young plants and blossoms than Bordeaux and hence produces less yield reduction; (6) Addition of cotton-seed-oil emulsion to cuprous oxide spray reduces its phytocidal action and tendency to reduce yield.

*Studies on a Ring-spot Type of Virus of Tomato.* E. P. IMLE and R. W. SAMSON.

A tomato disease characterized by intricate patterns of brown necrotic rings and lines on young leaves, broad, sunken, necrotic streaks on petioles and stems of young shoots, necrosis of shoot terminals, and often corky brown necrotic rings on green and ripe fruits, occurs in many sections of Indiana. The symptoms suggest the name tomato ring spot. Infected tomato plants seem to recover from the disease but retain the virus in infectious form. High temperatures increased the severity of the disease. Mechanical transmission of the virus was best effected by using carborundum abrasive. Mechanical transmission from tomato to Jimson weed was easily effected, but transmission back to tomato was possible only by grafting, or by passage from Jimson weed to tobacco and thence to tomato. A thermal death point of 56° to 58° C. for 10 minutes has been established. Twenty-one to 27 hours aging *in vitro* at room temperature inactivated the virus. Dilutions of 1: 500 rendered the virus completely noninfectious. The tomato ring-spot virus has been transmitted to and recovered from 14 solanaceous species and one species in the Amaranthaceae.

*Aerial Bacterial Strands in Fire Blight.* S. S. IVANOFF and G. W. KEITT.

Aerial bacterial strands on pear blossoms, young fruits, and shoots inoculated with *Erwinia amylovora* were observed and subsequently repeatedly produced experimentally in the greenhouses and the field in the spring of 1936. These strands are hair-like, more or less curved, usually colorless and glistening, and composed of cells of the blight pathogen bound together by a cementing substance. Their length varied from a fraction of a millimeter to several centimeters, their width from 8 to 45  $\mu$ . They disintegrate in glycerine slowly and in water instantly, releasing a great number of viable and infectious bacteria. They appear to be a special form of the well-known bacterial exudate, their component materials originating in the internal diseased tissues and emerging at the surface through minute openings. They are easily broken off and disseminated by the wind. Their rôle in the epidemiology of fire blight is yet to be determined.

<sup>1</sup> Cooperative investigation between the Agricultural Experiment Station, Geneva, N. Y., and the Cuprocide Fellowship of the Crop Protection Institute.



*Experimental Spraying for Combined Control of Apple Scab and Fire Blight.* G. W. KEITT and J. B. CARPENTER.

The following materials were used, with or without supplements: Bordeaux, 2-6-100; Coposil, 2 to 4-100; copper oxalate, 2 to 4-100; copper phosphate-lime-bentonite, 4-8-4-100; Cuproicide, 1½-100; lime-sulphur, 2-100; lime-sulphur, 2-100, with Bordeaux, 2-6-100, substituted in applications 1, B, and 2; lime-sulphur, 2-100, plus hydrated lime, 4-100; and lime-sulphur, 2-100, plus ferrous sulphate, 2 to 4-100. The applications, adapted to the unusual season, were: (D) delayed dormant, (1) open cluster, (B) full bloom, (2) petal fall, (3) 10 days later, (3A) 25 days after 2. Each material was used on at least two plots, each containing approximately 24 20-year-old Wealthy trees in the "off-year," about one-third in full or partial bearing. There was moderate blossom blight, little twig blight. Lime-sulphur gave no blight control: lime-sulphur plus supplements, little. The 12 copper-sprayed plots averaged about one-fifth as much blight as the 8 sulphur-sprayed. The mixed program was as effective as any. Little russetting occurred. There was not enough scab for satisfactory test of its control. The results indicate possibilities for a combined scab and blight spray program, but do not justify recommendations.

*Eradicant Fungicides in Relation to Apple-Scab Control.* G. W. KEITT and D. H. PALMITER.

In small-scale fall-spraying experiments, an application of certain copper-lime-arsenite sprays to apple trees reduced the incidence of perithecia of *Venturia inaequalis* the following spring by 99 to 100 per cent, without serious host injury. A spring application of a pound-to-gallon aqueous solution of ammonium sulphate to overwintered apple leaves on the ground killed the mature ascospores of the scab fungus and prevented its further development. Effects of eradican treatments on scab epidemiology were studied. In an orchard, fall-sprayed with copper-lime-arsenite mixtures in 1935, overwintered leaves averaged 5 perithecia per square inch, compared with 255 in a similar nonsprayed orchard some 300 feet away. Neither orchard was summer-sprayed in 1936. Counts showed the following percentage reductions in the incidence of scab lesions per leaf or fruit in the fall-sprayed orchard, based on comparative data from the unsprayed: *leaves*, Wealthy, June 2, 99, July 6, 87; Northwestern Greening, June 4, 94, July 9, 85; *fruit*, Wealthy, July 10, 87, Sept. 2, 75. At harvest, Sept. 2, the untreated orchard had 99 per cent of Wealthy fruit scabbed, the treated 55. Northwestern Greening bore insufficient fruit for counts.

*The Use of Movies in Teaching Symptoms and Control Measures of Cabbage Diseases.* KIRBY, BURKE and BAUER.

*Contaminated Soil in Relation to the Epiphytology of Tobacco Mosaic.* S. G. LEHMAN.

Studies are being made to determine the relation of contaminated soil to the epiphytology of tobacco mosaic. Four plots were laid out in a field of Norfolk sandy loam. Two of these plots were in tobacco in 1935, approximately 100 per cent of the plants having mosaic. The entire crop of diseased tobacco was disked into the land at the end of the growing season. The other 2 plots were in corn in 1935. Mosaic-free tobacco plants were set on all the plots in 1936. Up to topping time, no mosaic appeared on the tobacco where corn had grown. Only 0.65 per cent of the plants became diseased where the mosaic tobacco had grown. Some additional mosaic developed after topping. It is believed that the greater part of this resulted from accidental handling of a diseased plant in topping.

These results are in harmony with others already published and confirm the belief of the writer that only a very small percentage of tobacco plants becomes diseased as the result of the direct passage of mosaic into the plants from the soil, even though the soil be heavily contaminated.

*Crown Gall on Nicotiana glauca and Nicotiana langsdorffii and the Spontaneous 'Tumors' of Their Hybrid.* MICHAEL LEVINE.

*Nicotiana glauca* and *Nicotiana langsdorffii* do not respond with the same vigor to the tumor-inciting activity of *Bacterium tumefaciens*.

Inoculation of the crown of *N. glauca* induces large, massive, benign overgrowths that remain active for many months. *Nicotiana langsdorffii* inoculated with *B. tumefaciens* produces small tumors that do not live for more than 2 or 3 months. Microscopical studies of these overgrowths show the characteristic histological structure of crown gall.

Spontaneous tumor-like structures on stems and roots of the hybrids derived from crossing *Nicotiana glauca* and *N. langsdorffii* have been studied. Macroscopically, some of these overgrowths in their early stages of development have the appearance of crown galls. Microscopically, these growths differ from crown gall in that they are composed of numerous *anlagen* of stems and leaves. The growths that appear on the upper branches of the plants consist of proleptic shoots, many of which are fasciated. The growths are not invasive nor do they seem to interfere with the life of the plant. These so-called spontaneous tumors of this *Nicotiana* hybrid are organoid and are comparable with teratomas in animals.

*A Growth Hormone in the Development of Crown Gall.* S. B. LOCKE, A. J. RIKER, and B. M. DUGGAR.

Tomato plants inoculated with a virulent strain of *Phytopomonas tumefaciens* exhibit, in addition to gall development, responses that indicate the presence of a growth hormone in greater concentration than is present in similar, but noninoculated, plants. These responses include (1) increased epinasty of leaf petioles, (2) increased initiation of root primordia on the stem, (3) stimulated cambial activity, (4) inhibited development of axillary buds, and (5) delayed formation of an abscission layer in petioles of senescent leaves. When an attenuated culture was introduced into tomato stems, gall development was much less when growing tips, or expanding leaves were absent than when they were present. Crown galls induced by a virulent culture had an effect similar to growing tips.

*Inactivation of Tobacco-mosaic Virus by Ascorbic Acid.*<sup>1</sup> MARY LOJIKIN.

The reduced form of ascorbic acid in concentrations as low as 0.03 mg. per cc. can produce complete inactivation of purified preparations of the virus of tobacco mosaic. Inactivation takes place only when the ascorbic acid in the virus solution undergoes oxidation by atmospheric oxygen. Conditions that prevent the auto-oxidation of ascorbic acid or decrease its rate prevent inactivation or decrease the rate of inactivation of the virus, while the addition of copper, which catalyses the auto-oxidation, stimulates the inactivation. The virus remains active when ascorbic acid is oxidized in the absence of atmospheric oxygen by such oxidizing agents as iodine, 2,6 dichlorophenolindophenol, and potassium permanganate.

Dehydroascorbic acid does not produce inactivation of the virus under conditions resulting in inactivation by the reduced form. The virus in the whole juice of tobacco plants is less readily inactivated than the purified preparation.

Tomatoes from healthy and diseased plants, grown under the same conditions, are equal in vitamin C content.

*Comparative Properties of Virus Proteins from a Single-lesion Strain and from Ordinary Tobacco-mosaic Virus.* H. S. LORING and W. M. STANLEY.

Tobacco plants, *Nicotiana tabacum*, infected with virus derived from ordinary tobacco mosaic by several passages in *N. glutinosa* plants by means of single necrotic

<sup>1</sup> This article was preprinted November, 1936, from Contributions from Boyce Thompson Institute, Vol. 8, No. 4, 1936.

lesions, develop symptoms of typical tobacco-mosaic disease. When these plants are treated by the procedure used for the isolation of virus protein, a crystalline preparation closely resembling that from ordinary tobacco-mosaic virus, but differing in certain respects, is obtained. The single-lesion virus protein has approximately the same crystalline form as the ordinary tobacco-mosaic virus protein, but the crystals are somewhat longer and narrower. The two proteins have the same general properties and the same optical activity and isoelectric point. The virus protein from the single-lesion strain has a higher sedimentation constant than does that from ordinary tobacco mosaic, and is also considerably less soluble under comparable conditions. Both proteins have solubilities characteristic of solid solutions rather than of single homogeneous substances. The results obtained from solubility determinations and from ultracentrifugal analysis indicate that the virus protein from the single-lesion strain is more homogeneous than that isolated from plants infected with the ordinary strain of tobacco-mosaic virus.

*An Iris Leaf Disease Caused by Bacterium tardicrescens* n. sp. LUCIA McCULLOCH.

Affected leaves show translucent spots of irregular shape and usually of considerable area. In severe infections the lesions coalesce and cover the entire leaf. In early stages they are dark green, by reflected light, later turning yellow to brown. A period of continued moist weather appears to be essential for serious infection. Under dry conditions infection fails entirely or results in small, inconspicuous spots. Rhizomes are not affected.

The bacterial nature of this disease has been established by the usual methods of isolation and inoculation. The organism is a rather difficult subject for study because of its slow and often erratic growth both in the host and in culture media. On culture media the growth is yellow. The bacteria are motile by means of a single polar flagellum; have inconspicuous capsules; are Gram-negative; and are not acid-fast. Various other characters definitely separate the organism from described pathogens. Because of its slow growth the name, *Bacterium tardicrescens* is suggested.

*Isolation of Pathogenic Variants from Pure Cultures of Bacterium stewartii*. GEORGE L. MCNEW.

Differences in pathogenicity of pure cultures of *Bacterium stewartii* have been determined by the average number of necrotic lesions per leaf produced by inoculation of 10-day-old Golden Bantam sweet-corn plants. Pathogenic strains derived as single-colony isolates from sparsely seeded dilution plates of pure cultures and from inoculated plants have been studied.

Although a pure culture did not change appreciably in regard to pathogenicity within a year, variants were derived from it by single-colony isolation, which included types both more and less virulent than the parent culture. Two of these variants, which repeatedly gave 0.02 and 1.00 lesion per leaf in inoculation tests extending over a period of one year, in turn gave other variants by single-colony isolation. Variants with all degrees of pathogenicity were isolated from inoculated plants. The majority of the variants isolated from a plant inoculated with a virulent strain were similar to the strain used for inoculation. The proportion of extreme variants from this virulent culture was decreased by host passage. The majority of the variants isolated from a plant inoculated with a weakly pathogenic culture was more virulent than the original culture.

*Didymellina poecilospora*, n. sp., a Semiparasitic *Heterosporium* on Bulbous Iris. FRANK P. MCWHORTER.

The overwintering foliage of bulbous iris in the Pacific Northwest is frequently discolored by a *Didymellina* that forms a conidial stage entirely distinct from *Heterosporium gracile*. This species forms conidia abundantly only in newly infected leaf

areas. The conidial stage soon ceases and is followed by abundant development of perithecia. The black discoloration of the leaves is due to the profuse perithecial formation. Perithecial structures are identical with *Didymellina iridis* except for size. Ascospores average  $6 \times 25 \mu$ . Conidial stage a very variable *Heterosporium*. Conidia on host  $6-9 \times 12-38 \mu$ , typically bicellular. In wet weather, on host or in damp chamber or agar cultures, the conidia proliferate and assume a *Cladosporium* habit. The name *Didymellina poecilospora* is proposed for this mildly parasitic species, which should not be confused with the virulent *Heterosporium gracile*.

*Control of Rhizoctonia with Aniline Dyes.* JOHN MONTEITH, JR.

Experiments conducted at Arlington Farm, Virginia, during the past summer indicate that certain aniline dyes afforded effective control of turf diseases, particularly large brownpatch (*Rhizoctonia solani*). Malachite green and crystal violet gave good control. Auramine O controlled the disease but was much less effective than the preceding. Rates varied from  $\frac{1}{16}$  to  $\frac{1}{2}$  ounce per thousand square feet. The heaviest rates were required when the weather conditions were particularly favorable for the fungus. Promising control of *Pythium* was observed. The dyes checked dollarspot, but failed to give satisfactory control of severe attacks. The dyes proved less effective than corrosive sublimate applied at equal rates. However, they had a distinct advantage over all the mercury fungicides in causing no injury to grass at the heaviest rates. The color of all of these dyes proved objectionable. They were, therefore, combined in such proportions as to give a dye that closely matched the color of healthy grass. Such combinations did not affect the fungicidal properties of the separate ingredients and, in addition to giving disease control, served effectively to mask all moderate disease attacks.

*Comparison of Enzymes in Grown-Gall and Noninoculated Plant Tissue.* R. NAGY, W. H. PETERSON and A. J. RIKER.

Certain enzymes found in mature crown-gall tissue and in contiguous noninoculated tissue have been examined. Quantitative determinations of oxidase, peroxidase, and catalase yielded 130, 120, and 160 per cent greater enzyme activity, respectively, in the fresh gall tissue than in the contiguous noninoculated tomato tissue. A more striking difference was found in the tyrosinase content of the two tissues. Fifty cc. of expressed crown-gall juice destroyed in 10 hours half of the tyrosin in 200 cc. of a 0.05 per cent solution. No loss was detected from a similar preparation of noninoculated tomato stem tissue.

*A Disorder of Cotton Plants Recently Observed in Louisiana.* D. C. NEAL.

A disorder of cotton plants, resembling in certain respects the symptoms caused by some of the mosaic diseases, has been observed at the Louisiana Experiment Station since 1934. The disease has appeared regularly in portions of a 3-acre tract in a soil classified as Lintonia silt loam, the affected plants becoming discernible within 35 to 40 days from time of planting. The first noticeable feature is the appearance of the leaves of affected plants. These are puckered, mottled, and variously distorted in the early stages with necrotic lesions appearing along and between the veins; and later, as the plants approach maturity, they become slightly thickened and ragged at the margins. Fasciation of branches, with reduction in size of involucre bracts and floral buds, also occurs, resulting in the formation of small, unsymmetrical bolls. Shedding of flower buds and young bolls, however, is not characteristically associated with the disorder, as is the case with plants affected with crazy top.

The disease has been observed in several varieties of upland cotton. Efforts to transmit it to healthy plants by injecting sap from various parts of diseased plants were unsuccessful, and negative results also were obtained in top- and in arch-grafting experiments.

*Verticillium Wilt of Peppermint.* RAY NELSON.

A serious wilt of peppermint is prevalent in Michigan and also occurs in other States. It was first observed in 1924, but the earlier abandonment of large acreages is traceable to this disease. Initial symptoms seen in May or June include: (1) dwarfing; (2) unilateral development of terminal leaves; (3) bronze color in terminal leaves. In July and August infected plants show a typical *Verticillium* syndrome and succumb rapidly. Premature cutting of affected fields is necessary and results in a low yield of oil. English and American peppermint are very susceptible, but some of the spearmints are resistant. Wilt is caused by a species of *Verticillium* morphologically similar to *V. dahliae*. Soil moisture has a striking influence on the development of the disease and excessive drainage and droughts have increased its destructiveness during the past 6 years. Good commercial control has been achieved by maintaining a high water table. Work is in progress in an attempt to produce resistant mints by hybridization.

*Basal Dry Rot of Gladiolus Corms.* RAY NELSON.

A dry rot affecting the basal portion of gladiolus corms has been observed for several years and is increasing in prevalence in commercial stocks. The lesions occur mostly on the first and second internodes but may involve other areas. At first the lesions are small, sunken, round or elliptical with a sharply limited border. By enlargement and confluence the entire basal portion of the corm may become involved. At harvest time the affected areas are dark brown to black, rough and scaly, and penetrate the corm to a maximum depth of about 3 mm. Progress of the decay is checked by drying the corms; decay does not continue unless there is delay in cleaning and curing. Planting infected corms results in poor stands due to failure to form roots or to further decay. Proof of the causal relation of *Fusarium* to the disease has been obtained in inoculation experiments in the greenhouse and field. Treatment of infected corms with calomel or yellow oxide of mercury, 1 pound to 5 or 10 gallons of water, has given good control.

*Blue Stain of Cotton is Due to a Fungus.* O. P. OWENS.

Recent studies by the writer have shown that at least one fungus will produce blue stain, thereby sometimes greatly lowering the market value of cotton. A species of *Alternaria* isolated from cotton affected with blue stain has repeatedly caused the natural blue stain, when it was placed on sterile mature cotton fibers. Staining was produced much more rapidly in cotton taken from apparently mature green bolls, occurring as early as 5 days after the inoculation and continuing until the whole lock was completely blue. Locks of green bolls inoculated in the field showed definite staining 15 days after inoculation. Other locks in the same boll, but not inoculated, finally developed the blue stain after the causative fungus had spread to them from the inoculated locks.

*An Undescribed Potato Disease in West Virginia.* C. R. ORTON and L. M. HILL.

The disease was first noted in the Appalachian region about 6 years ago, since when it has spread so rapidly as to have become a limiting factor in production in certain districts of West Virginia.

The first external symptoms are characterized by a dwarfing, paling, and upward folding of the terminal leaflets. Within 7 to 10 days the vines wilt and die. The vascular region of the stems, tubers, stolons, roots, and numerous spots in the stem pith turn brown. A discontinuous dendritic necrosis of the stem end of the tuber is characteristic.

An extensive necrosis exists in the phloem and adjacent parenchyma, similar to that in leaf roll and net necrosis, and to a lesser extent in the xylem. A granular de-

posit occurs in the necrotic areas, which sometimes show lysigenous cavities. The vessels are infrequently filled with similar granular material.

The necrotic areas in tuber parenchyma lose their starch; standard tests for lignin and pectin indicate the disappearance of these compounds from the diseased areas. Suberin appears to be found in walls of surrounding cells.

The etiology of the disease is unknown. It does not seem to be transmitted through the tuber.

*Studies of Copper-Lime-Arsenite Dusts for Control of Wheat Bunt.* D. H. PALMITER and G. W. KEITT.

Potentialities of copper-lime-arsenite dust mixtures for seed treatments were studied in relation to control of bunt. In 5 series of greenhouse experiments these preparations were tested in comparison with commercial copper carbonate and ethyl mercury phosphate dusts. When bunt-infested seed was treated and planted in clean soil, all the dusts used gave practically complete control. When clean or infested seed was planted in infested soil, certain copper-lime-arsenite dusts consistently gave slightly better control than the commercial materials. The copper-lime-arsenite preparations increased the percentage of germination of the wheat seed more than the copper carbonate, but less than the mercurial dust. Limited field experiments at Madison gave results similar to those obtained in the greenhouse. These experiments show that the copper-lime-arsenite preparations have a comparatively high fungicidal value under the conditions encountered.

*Marigold Wilt.* P. P. PIRONE.

A severe outbreak of wilt on marigolds, *Tagetes erecta*, caused by *Phytophthora* sp. occurred last summer in the metropolitan New York area. The disease first appeared early in June on half-mature plants of the variety Guinea Gold. Conspicuous leaf wilting was visible. An area on the stem extending from the crown to several inches above the soil surface was blackened and sunken. Plants growing under moist conditions exhibited stem discoloration extending into the pith, while those growing in drier soil showed a brownish discoloration, principally in the cortex, with very little infection in the woody parts. Inoculations by wounding stem bases, adding inoculum to soil and to wounded roots produced symptoms typical of the disease. Young plants thus inoculated were killed within 6 days. Half-grown to nearly mature plants succumbed in from 10 to 21 days. Of the varieties of African marigolds inoculated, Guinea Gold was the most susceptible. The causal organism was reisolated in every case of successful infection in the greenhouse. Inoculation of the stem bases in a few of the dwarf marigolds failed to produce infection. A species of *Fusarium*, often associated with wilt, was found to be nonpathogenic. Under greenhouse conditions control of marigold wilt was readily obtained by steam sterilization of the soil. Several fungicides applied to infested soil also offered some degree of control.

*Classification of Lily-mosaic Virus.* W. C. PRICE.

Three strains of cucumber-mosaic virus were transmitted to *Lilium longiflorum*. They produced symptoms similar to those of lily mosaic in this host. Virus from diseased lilies, obtained from a commercial grower, was transmitted to *Nicotiana tabacum*. It caused primary necrotic lesions and remained localized, producing only an occasional systemic lesion. On passage from tobacco to tobacco it gave rise to a strain that became systemic and produced mottling symptoms. This passage strain was transferred to *Zinnia elegans*, in which it produced mottling symptoms. Inoculation experiments have demonstrated that zinnia leaves, thoroughly invaded by the passage strain of lily-mosaic virus, are immune from infection with virus of cucumber-mosaic strain 6. It has previously been shown that zinnia leaves mottled by cucumber-mosaic virus, or strains of this virus, are immune from infection with virus of cucumber-mosaic strain 6, and that infection of zinnia plants with viruses unrelated to that of cucumber mosaic

does not protect them from infection with cucumber-mosaic strain 6 virus. It is, therefore, concluded that lily-mosaic virus should be classified in the cucumber-mosaic virus group.

*Root Rot of Rice.* T. C. RYKER.

A root rot of rice, associated with a species of *Pythium*, was observed in several localities in Louisiana in 1936. In general, the disease occurred where growth conditions were not satisfactory, principally in alkali spots and in areas where the fertility of the soil was poor. In the alkali spots the plants became chlorotic and the roots decayed rapidly, showing the typical flaccid root tips characteristic of most *Pythium* root rots. When such fields were drained the plants produced new roots, and many of these survived after the fields were reflooded. In fields showing low fertility, particularly along terraces where the soil was thin, the plants after flooding became stunted and in some instances died. The stunted plants would wilt during the hot part of the day because of the poor decayed root systems, but no chlorosis occurred. Draining these fields did not affect the surviving plants. Some of the plants partially recovered, whether the fields were flooded or drained. Inoculation experiments have not shown the *Pythium* to be pathogenic when the plants were growing satisfactorily.

*Methods and Results of Studying Some of the Factors Involved in Spray Injury of the Apple.* F. J. SCHNEIDERHAN.

Seventy-five different applications of most of the common fungicides and insecticides were made with a hand sprayer at 130 pounds' pressure under specially selected weather conditions hitherto considered to be conducive to spray injury. Comparisons of high- and low-pressure applications indicated that high pressure is a predisposing factor to spray injury. A complete history of so-called "sulphur shock" resulting in leaf paralysis and leaf dropping after 25 days was recorded. Delayed action of both copper and sulphur injury was observed. Data on the time factors involved in spray injury during certain weather conditions have been recorded. At a temperature of 105° F. pure water sprayed on apples very susceptible to spray injury failed to cause injury. Observations regarding the time required for complete drying of different fungicides indicate a minimum of 15 minutes under favorable conditions to 3 hours under conditions of high humidity. A study of the physical appearance of fruit russet caused by different spray materials indicates that, with only one exception, russet is essentially the same. A type of russet designated as "black pinhead russet" was typical of Bordeaux injury on certain varieties. All of the copper-containing fungicides caused russet under certain weather conditions. Combinations of lime-sulphur 1-80 and half the usual amount of certain wettable sulphurs cause more sulphur burn than standard amounts of these fungicides used alone.

*Basal Decay in Oak Stands of Sprout Origin.* BAILEY SLEETH and ELMER R. ROTH.

Extensive field studies in cooperation with forest agencies and Civilian Conservation Corps have been made in the Eastern and Central States to determine the sources and extent of basal decay in oaks of sprout origin, factors contributing to decay, and practices necessary to reduce future decay losses. Basal decay was found to present a greater hazard in sprout trees than in seedlings or seedling sprouts because of the danger of decay being transmitted to the sprout from the parent stump and from dead stubs of companion sprouts. Approximately 25 per cent of the trees examined were decayed, *Quercus velutina* showing the most and *Q. montana* the least infection. *Stereum gausapatum* was determined by cultural studies as the causal organism in over 75 per cent of the cases. Over 90 per cent of the decay was traced directly to the parent stump, the remainder originating largely from the removal of a companion sprout or from a dead standing sprout. Factors



concerned with high and low decay hazard were diameter and height of the parent stump, height of sprout origin, presence of stump wounds, the time and condition of heartwood unions, and the presence of dead stubs of companion sprouts. Certain forest practices that, if followed, should serve to reduce future losses in sprout stands, were indicated.

*Studies on the Host Range of Bacterium solanacearum.* T. E. SMITH.

*Bacterium solanacearum* E.F.S., the cause of the Granville wilt of tobacco, attacks numerous cultivated and wild species of plants. The disease is soil-borne and, consequently, the wide host range greatly complicates control by rotation. Since many reports of host species have been based entirely on successful stem inoculations, a study has been made of susceptibility as indicated by this method of inoculation as compared to susceptibility as indicated by growing the plants in a badly infested field. All plants susceptible to natural infection also proved susceptible to stem inoculation, but the reverse was not always true. Out of 61 species tested, 5, including the important legumes, velvet beans, soybeans, and cowpeas, were susceptible to the artificial inoculation but apparently immune from natural infection. These data raise the question as to whether species immune from natural infection can be regarded as host plants.

*Relation of Injuries to Infection of American Elm by Ceratostomella ulmi.* S. J. SMUCKER.

Infection studies with 5- to 8-foot American elms in the greenhouse indicate that *Ceratostomella ulmi* probably cannot invade the vascular system unless injuries through the bark are present. Numerous attempts to infect young trees with *C. ulmi* by placing the inoculum on uninjured surfaces or in injuries in the cortex of roots, trunks, branches, twigs, succulent shoots, and leaves were unsuccessful. However, infection was readily secured under similar conditions when the inoculum was placed in the following types of injuries extending to the xylem: Tangential knife incisions in the roots, trunks, or branches; wounds made by the removal of wedge-shape chips of bark and xylem from the trunk and branches; split branches; injuries in crotches of twigs; pruning injuries; torn petioles; and needle punctures into the wood of roots, trunks, branches, twigs, succulent shoots, and leaves. There are insufficient data to indicate whether leaf infections are a factor in the natural spread of the disease. Trees inoculated during the period the spring wood was being formed developed external symptoms more rapidly and in a higher percentage of cases than those inoculated late in summer.

*Influence of Nutrition on Systemic Development of a Yellow Strain of Tobacco Mosaic.* ERNEST L. SPENCER.

Investigations have been continued on the interrelationship between host nutrition and host response to virus infection. Seedlings of *Nicotiana tabacum* var. Turkish were grown in sand cultures and supplied with nutrient solutions in which the nitrogen, phosphorus, and potassium contents were varied in turn. After 4 weeks of nutrient treatment, representative plants were inoculated by rubbing the tip of a leaf situated about half-way up the stem. The inoculum was nondiluted juice from a tobacco plant diseased with yellow tobacco mosaic (Johnson's tobacco virus 6). Growth was measured by recording the green weights of representative plants at time of inoculation. There was no apparent correlation between growth and the time of appearance of systemic infection. However, the time of appearance of secondary or systemic symptoms appeared to be definitely correlated with the nutrition of the host. Plants receiving nutrient solutions deficient in either phosphorus or potassium showed systemic symptoms much earlier than similarly inoculated plants receiving an excess of either phosphorus or potassium. Excess nitrogen brought about no appreciable delay in the appearance of systemic infection.



*Seasonal Cycle of Ustilago hordei.* V. F. TAPKE.

At least under conditions at Arlington Experiment Farm, Rosslyn, Va., the seasonal cycle of barley covered smut (*Ustilago hordei*) does not coincide with the generally accepted beliefs that barley covered-smut spores remain confined to the smutted heads until threshing; that inoculation occurs at threshing through adherence of spores to the surface of seed, and that the seed-borne spores remain dormant until seeding. At Arlington Farm, spore dissemination begins soon after emergence of the smutted heads from broken areas in membranes that enclose the sori and it continues through the ripening, cutting, shocking, and threshing of the grain. Of the spores that are blown, washed, or otherwise carried to the grain, some eventually come to lie beneath the hulls or send infection hyphae beneath the hulls or both. The positional advantage of sub-hull inoculum to infection of the seedling, in field-inoculated seed, doubtless explains why such seed with a relatively light spore-load, so frequently produces much higher percentages of smutted plants than seed that has been surface-inoculated by blackening with millions of spores. The occurrence of sub-hull inoculum also accounts for the previous difficulties in obtaining perfect control of this smut in plants from field-inoculated seed treated with surface disinfectants.

*Laboratory Studies on the Fungicidal Properties of Sulphur.* J. J. TAUBENHAUS.

Studies were continued on the fungicidal properties of sulphur when used alone or in combination with various forms of copper and some standard insecticides. The materials were tested in their ability to inhibit germination of spores of certain plant pathogens or saprophytic organisms that cause decay to perishables in transit or storage. It was found, as in previous work, that sulphur alone was highly toxic to the spores of certain microorganisms. In other cases, toxicity of sulphur was benefited by the additions of slight amounts of other standard fungicides. The addition of certain insecticides weakened the toxicity of the sulphur mixtures.

*Separation of Actinomyces Isolates Obtained from Scabbed Potatoes and from Soil.* C. F. TAYLOR.

A comparative study of Actinomyces isolates from scabbed potato tubers and from soil, using bacteriological methods, leads to the conclusion that separation within this group may be accomplished through physiological tests. Primary separation was based on partial acid-fastness (correlated in those studied with the inability to hydrolyze starch). Within the group of 128 non-acid-fast isolates, the pigment response in the surface ring on brom cresol milk, the presence of nitrites after good growth on Zobell's nitrate medium, the utilization of carbon compounds, the final reaction in milk; and the maximum temperature permitting growth gave separatory differences. Within the small partially acid-fast group (15 isolates), the inability to liquefy gelatine was correlated with microaerophilic growth on nutrient agar shake tubes, and provided separation into two main groups. Succinic, formic, oxalic, and citric acids have been more useful than any other of the 30 carbon sources tested. Through these means the 143 isolates studied were separated into 55 different species. So far, no species names have been applied in this work, since similar studies are being conducted on named species obtained through S. A. Waksman and from Baarn.

*Inheritance of Resistance of Barley to an Undescribed Physiologic Form of Erysiphe graminis hordei.* J. S. TIDD.

Studies of  $F_2$  and  $F_3$  of 3 crosses between resistant and susceptible barley plants were made in the greenhouse. An undescribed physiologic race of barley mildew, here designated as physiologic race 6, was used as inoculum. The resistance of the 3 resistant parent plants was found in each case to be due to a single main Mendelian factor. (1) In

the cross, Svansota M786 × Hanna C. I. 906, resistance was incompletely dominant, the heterozygous individuals being somewhat less resistant than Hanna and homozygous resistant segregates. (2) In the cross, Featherston C. I. 1118 × Goldfoil C. I. 928, resistance was again incompletely dominant. In the  $F_2$  test, conducted in the late spring in the greenhouse, heterozygous individuals gave a reaction of type 2-3, intermediate between resistance and susceptibility. In the winter, however, heterozygous  $F_2$  plants were more resistant and gave 1-2 reactions. Independent inheritance of the factor pairs for resistance versus susceptibility and 2-row spikes versus 6-row spikes was indicated. (3) In the cross, Arequipa C. I. 1256 × Horsford C. I. 610, resistance was dominant. Independent inheritance of the factor pairs for resistance versus susceptibility and hoods versus awns was suggested.

*Reaction of Barley to Two Undescribed Physiologic Races of Barley Mildew, Erysiphe graminis hordei.* J. S. TIDD.

The reactions of 85 varieties of barley in the seedling stage in greenhouse studies to 2 new physiologic races of *Erysiphe graminis hordei* were studied. These 2 races are designated as physiologic races 6 and 7. In order to separate races 6 and 1, another barley variety, Heil's Hanna 3 C. I. 682, was added to the list of 4 differentials used by Mains and Dietz in their studies. Races 6 and 7 were tested on seedlings of a number of varieties during the winter and spring under the differing environmental conditions then prevalent in the greenhouse. No marked changes in reaction were shown to either race. Tests of varieties in the maturing stage in the greenhouse showed that such barley plants in the spring were more resistant to mildew than seedlings of the same varieties. Maturing plants in the winter were fully as susceptible as seedling plants of the same varieties.

*The Fungicidal Value of Mustard Oils.* J. C. WALKER.

In connection with a study of the possible relation of the mustard oil, allyl isothiocyanate, to resistance in certain crucifers to *Plasmodiophora brassicae* Wor. the fungicidal value of a number of volatile sulphur oils upon several facultative saprophytes was determined. Arranged in a descending order of toxicity, the substances studied are as follows: allyl isothiocyanate, phenyl isothiocyanate, methyl isothiocyanate, ethyl isothiocyanate, ethyl mercaptan, methyl mercaptan, methyl thiocyanate, ethyl thiocyanate, allyl sulphide, methyl sulphide, ethyl sulphide. The glucoside of allyl isothiocyanate, sinigrin, has little or no toxic value. The free oil completely prevents growth of *Colletotrichum circinans* when added to Czapek's medium at 30 parts per million, while no effect upon growth was noted upon the addition of 1000 parts or more per million of the oil in the glucoside form.

*Increasing Importance of Cabbage Mosaic.* J. C. WALKER and R. H. LARSON.

In 1935 and 1936 this disease increased in severity on cabbage, cauliflower, and flowering broccoli in southeastern Wisconsin, especially on late plantings, which were exposed to heavy infestation of cabbage aphid, the insect vector. Various manifestations of mosaic and necrotic patterns on outer foliage were followed commonly by internal "fly speck" necrosis throughout the head. Almost complete dropping of outer leaves reduced yield as much as 40 per cent. Continued abscission of inner head leaves caused an important storage loss and predisposed the tissue to secondary organisms. Seed productivity of affected plants was distinctly reduced in a large greenhouse planting.

*Pathogenicity of a Brown Cultural Variant of Ceratostomella ulmi.* JAMES M. WALTER and CURTIS MAY.

A study of single-spore isolates of *Ceratostomella ulmi* indicated that the species comprises a wide range of cultural races. Some of the strains appeared to retain their

cultural characteristics through several transfers; others continued sectoring. Variants have been noted among both monoconidial and monoascospore isolates.

Among the isolates recognized, a brown variant strikingly different from the characteristic type has been isolated from diseased trees in the United States and in England. American elms in the greenhouse and English elms growing naturally developed typical disease symptoms after inoculation with this brown variant. The variant was recovered from the inoculated trees in all cases.

*Penetration and Invasion of Phymatotrichum omnivorum in Cotton Roots Grown Under Pure-Culture Conditions.* G. M. WATKINS.

Roots of cotton seedlings grown in nutrient agar, inoculated with pure culture of *Phymatotrichum omnivorum* became rapidly overgrown with a mycelial web. Sections of such roots in various stages of invasion show that individual hyphae may penetrate the epidermal cell wall or root hairs. From the epidermis the hyphae grow through and between the cells of the cortex. Sometimes penetration of the host cells is accomplished by means of constricted hyphal tips of the fungus. The hyphae finally penetrate the endodermis and enter the stele, where longitudinal progression has been observed in the various tissues of the vascular cylinder. No definitely organized haustoria have been observed. Invaded cells are finally killed, and in later stages are almost entirely filled with hyphae.

*Antiseptic and Disinfectant Treatments of Flowering Bulbs.* FREEMAN WEISS.

Because of centripetal growth and frequent absence of wound periderms in surface tissues, flowering bulbs and corms are among the least adequately protected plant organs against pathogenic organisms. An increasing tendency to superficial infections, often eventuating in general necrosis and decay, is experienced in commercial culture of flowering bulbs when long continued in one site. The non-hygroscopic husks and cutinized surfaces of many bulbs introduce special requirements as to wetting, adherence, and penetration by antiseptics or disinfectants. Tests of materials commonly used for seed, soil, and tuber disinfection have been made on flowering bulbs for several years. Some botanical groups show a common toxicity relation with respect to certain chemicals; thus, bulbs of Amaryllidaceae are tolerant of mercury in any form and show vegetative stimulation by some forms; iridaceous bulbs are tolerant of most but not all forms of mercury; most liliaceous bulbs are very intolerant. Soluble copper salts are consistently toxic. Formaldehyde is safe with some mercury-intolerant bulbs; lime-sulphur is a generally safe and often efficient antiseptic. Certain bulbs, intolerant of soluble mercury and copper, were beneficially affected by dipping in suspensions of  $\text{HgO}$  and  $\text{Cu}_2\text{O}$  in aqueous emulsions of cottonseed and other oils stabilized with soap and other emulsifying agents.

*Noninfectious Chlorosis of Perennial Phlox and Its Relation to Phlox Blight.* FREEMAN WEISS and THELMA B. POST.

Blighting, without abscission, of the lower leaves is a symptom common to several pathological conditions that may affect perennial phlox. A distinct chlorosis, of virus-like pattern, observed in old phlox plantings, was suspected as a factor in the prevalence of blight under these conditions. Attempts to induce chlorosis in seedlings or other presumably disease-free phlox plants by mechanical methods of virus inoculation and by grafting failed. This chlorosis is not due to aster-yellows virus nor to any virus transmissible mechanically to tobacco, cucumber, or tomato. It appears in small and variable proportions of seed progenies, which explains its relative frequency in neglected plantings where seedlings are allowed to grow. It persists indefinitely through vegetative propa-

gation but symptoms are sometimes masked. Another pathological condition, characterized by rugosity and curling of leaves, proved noninfectious by the methods tried; its occurrence is limited to a few varieties. A third abnormality, manifested in necrotic spotting of foliage not associated with a demonstrable parasite was sometimes fatal within one season, or complete recovery occurred. None of these conditions is now considered a factor in phlox blight, which is attributed primarily to infection by *Septoria phlogis*, and secondarily to root injury by environmental factors such as high soil temperature and desiccation.

*Control of Damping Off of Conifers.* GEO. Y. YOUNG, W. C. DAVIS and D. H. LATHAM.

In preliminary nursery and greenhouse tests a crude phosphoric acid indicated effectiveness in the control of damping off. Results with a purer grade of the acid have been less satisfactory. Formaldehyde, applied to very sandy soil a week before sowing, seriously reduced emergence of jack pine when it was applied at the rate of  $\frac{3}{4}$  fluid ounce per square foot, but not where  $\frac{1}{4}$  ounce was employed. With black locust at another nursery both  $\frac{1}{4}$  and  $\frac{1}{2}$  ounce treatments applied after sowing resulted in decided increase in emergence. Ammonium compounds, as well as nitrates, have increased the damping-off hazard. Nursery-water supplies and sand introduced for covering seed at numerous nurseries, particularly in the central States, were unexpectedly found to cause a marked increase in the pH of the seed beds and thus in the damping-off hazard. Steps are being taken toward the correction of the pH of the water and the finding of more acid sand.

*Pea Streak and Its Relationship to Strains of Alfalfa Mosaic.* W. J. ZAUMEYER.

Three strains of the alfalfa-mosaic virus, infectious to pea, have been differentiated through cross-inoculation, host reaction, and property studies. The symptoms produced by these viruses on pea are similar to one another except in intensity. Strains 1 and 2 produce a leaf mottling, while strain 3 produces a leaf spotting in addition to a mottling. In 1935 the writer reported on a pea streak as being caused by an alfalfa-mosaic virus. It has since been proved that the pea-streak virus is distinct from the virus of alfalfa mosaic, although the symptoms produced by both on peas and horse beans are somewhat similar. No variety of pea thus far tested has shown resistance to the streak virus, while Horal variety is resistant to the 3 strains of alfalfa-mosaic virus. The host range of the streak virus is very limited, while that of the alfalfa viruses is not. The pea streak virus is infectious at 1-5000 dilution, while the alfalfa viruses lose their activity at 1-3000 dilution. The former is not active after 25 hours' aging, while the latter retain their activity as long as 5 days. The pea streak virus is killed at 65° C., while the alfalfa viruses are killed at 70° C.

# DISTRIBUTION AND PREVALENCE OF OZONIUM ROOT ROT IN THE SHELTER-BELT ZONE OF TEXAS

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## INTRODUCTION

For almost 50 years ozonium root rot has been the major disease of tap-rooted plants in certain areas of the Southwest. All investigators now are agreed that Ozonium has been indigenous to these areas for years, but for the most part its presence is manifested only when native vegetation is cleared off and the land is planted to susceptible crops. The range of Ozonium appears to be increasing each year in proportion to the amount of new land opened for cultivation, not only in Texas but in other areas throughout the Southwest.

This soil-inhabiting fungus attacks a greater number and variety of endemic and exotic plants than any other parasitic organism in this region. Regardless of the continual efforts to control root rot, it yet remains the most destructive disease in the Southwest and is responsible for large losses of cotton, alfalfa, and many other important crops, as well as for the death of many fruit and ornamental trees. No satisfactory methods of complete prevention have been devised, although its ravages can be inhibited either by planting nonsusceptible fibrous-root crop plants or by clean fallow or through manurial and chemical treatments. Furthermore, no evidence was found in the literature that the fungus has ever entirely disappeared from an infested area.

In view of the insidious nature of ozonium root rot, it was deemed imperative in advance of planting to map in detail the root-rot-infested areas within the Shelter-belt Zone in southern Oklahoma and Texas, the object being to avoid these areas or to plant resistant tree species. The survey was begun in early June, 1935, when the fungus could be detected in non-cropped lands without dependence on susceptible crop plants as indicators of the presence of the fungus. It is the purpose of this report to discuss the methods employed in determining the presence of Ozonium and to offer a preliminary map indicating the distribution and prevalence of root rot in the Shelter-belt Zone of Texas.

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<sup>2</sup> The writer wishes to acknowledge the assistance of Ernest Wright, Associate Pathologist, and F. R. Schroeder and M. M. Evans, formerly Field Assistants, Division of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture. He also desires to thank F. R. Schroeder for the photographs reproduced in this publication.

## DISTRIBUTION OF ROOT ROT IN TEXAS AND THE SOUTHWEST

In 1923 Taubenhaus and Killough<sup>3</sup> reported that ozonium root rot had been found, either by actual field surveys or through diseased specimens of plants sent in for identification, in 67 counties, located principally in east-central Texas. At that time no root rot in the 23 counties in the Shelter-belt Zone had been reported. Eight years later (1931) Taubenhaus and Ezekiel<sup>4</sup> listed 196 counties in which root rot had been observed, either in the field or in plants sent in for identification. Fifteen of the 23 Shelter-belt counties were listed by them as infested with root rot, but no data as to the exact extent of the disease were given for any of these counties.

At first thought it would appear that during the interval 1923-1931, Ozonium had spread rapidly westward from the waxy black lands of central Texas, but more intensive surveys and the extension of agriculture to new land merely revealed that Ozonium, already indigenous, had made itself evident when it attacked susceptible crop plants. A good illustration of this was observed by the writer in the valley of California Creek in north-western Jones County, where several hundred acres of mesquite land had been cleared the year before, plowed, and planted to cotton in June. At the time this field was visited, the cotton was small, and no root rot was evident; yet, weeds along the fence rows were dying from ozonium root rot; and conidial mats were plentiful on the sides of a road ditch under mesquite trees. It is easy to foresee that cotton cannot be profitably grown for any length of time in this ozonium-infested field.

A preliminary survey of the Shelter-belt Zone in southwest Oklahoma indicated that root rot was prevalent in certain areas in Tillman and Kiowa Counties. From earlier reports it is apparent that the fungus may be found in the two lower tiers of counties to the east and adjacent to the Red River in Oklahoma, since root rot has been noted repeatedly as far east as Miller County, Arkansas. In New Mexico, Arizona, and southern California, root rot has been reported primarily from some of the irrigated valleys. As recently as 1933, Richards<sup>5</sup> found Ozonium causing extensive damage to alfalfa and other susceptible crop plants to Washington County, Utah, in the Virgin River Valley, which empties into the Colorado River above Boulder Dam. Occasional reports also have indicated the presence of root rot in some of the irrigated sections of northern Mexico. Its natural range, therefore, extends from southwestern Arkansas and eastern Texas to southern California, and from southwestern Utah into Mexico.

<sup>3</sup> Taubenhaus, J. J., and D. T. Killough. Texas root rot of cotton and methods of its control. Texas Agr. Expt. Sta. Bull. 307. 1923.

<sup>4</sup> Taubenhaus, J. J., and W. N. Ezekiel. Cotton root-rot and its control. Texas Agr. Expt. Sta. Bull. 423. 1931.

<sup>5</sup> Richards, B. L. Phymatotrichum root rot discovered in Utah. U. S. Dept. of Agr. Bur. Plant Indus. Plant Disease Reprtr. 17: 36. 1933. [Mimeographed.]

Judging from the evidence collected by several observers during the past 10 years in many widely scattered localities in the Southwest, it can be definitely concluded that *Ozonium* is there indigenous, since typical strands of the fungus have been found in desert areas and in virgin lands on the roots of many endemic trees, shrubs, and plants, in some instances without subsequent injury and in others producing injuries sufficiently extensive to cause the death of plants. Where *Ozonium* is present on the roots of living or dead native plants, root rot usually follows on susceptible crops planted after the land has been cleared. In addition, conidial mats have been observed, where sufficient moisture was present, in strictly desert regions and on non-cropped lands far removed from cultivated areas; and lastly, sclerotia of the fungus have also been found in these same sections.

#### HOST PLANTS

Taubenhaus, Dana, and Wolff,<sup>6</sup> and Taubenhaus and Ezekiel<sup>7</sup> list upwards of 600 plant species which they have found to be susceptible in varying degrees to ozonium root rot. Roughly, about half of the total number consists of cultivated plants including field, truck, fruit, nut, and berry crops, as well as shade and forest trees, ornamental shrubs, and herbaceous plants, while the remaining half is composed of plants not ordinarily cultivated, such as many of the annual and perennial weeds, and native herbs, shrubs, and trees.

About 41 species of trees and shrubs, most of which are endemic to Texas and Oklahoma, have been recommended as suitable for planting in the Southern Section of the Shelter-belt Zone.<sup>8</sup> These are listed in table 1 on the basis of their reaction to root rot, according to the findings of Taubenhaus and his coworkers.<sup>6, 7</sup>

#### DESCRIPTION OF THE FUNGUS

The fungus causing root rot may be recognized by 3 distinct stages. There is, first the sterile mycelium (*Ozonium omnivorum* Shear), usually found in the form of buff-color strands, composed of one or more large central hyphae and surrounded by a varying number of small, irregular, thick-wall hyphae. From the more or less triangular cells in the outer band of the hyphae, the characteristic short, upright, acicular hyphae arise. The function of these strands appears to be that of advance mycelia, which spread out

<sup>6</sup> Taubenhaus, J. J., B. F. Dana, and S. E. Wolff. Plants susceptible or resistant to cotton root rot and their relation to control. Texas Agr. Expt. Sta. Bull. 393. 1929.

<sup>7</sup> Taubenhaus, J. J., and W. N. Ezekiel. Check list of diseases of plants in Texas. Texas Acad. Sci. 16: (1931-32), 5-89. 1933.

<sup>8</sup> Olsen, D. S., and J. H. Stoeckeler. The proposed tree plantations—their establishment and management. In: U. S. Forest Serv. Lake States Forest Expt. Sta. Possibilities of shelterbelt planting in the plains region. P. 15-27, 1935.

TABLE 1.—The relative susceptibility of tree and shrub species, recommended for planting in the Shelter-belt Zone of Texas, to ozonium root rot

Group and species	Non-sus- ceptible	Highly resistant	Moderately resistant	Susceptible	Highly susceptible
<b>Hardwoods:</b>					
Cottonwood, <i>Populus</i> sp. ....					X
Sycamore, <i>Plantanus occidentalis</i> L. ....		X			
Dwarf Asiatic elm, <i>Ulmus pumila</i> L. ....				X	
Honeylocust, <i>Gleditsia triacanthos</i> L. ....					X
American elm, <i>Ulmus americana</i> L. ....				X	
Russian mulberry, <i>Morus alba tatarica</i> (L.) Loud. ....				X	
Paloblanco, <i>Celtis reticulata</i> Torr. ....		X			
Osage-orange, <i>Toxylon pomiferum</i> Raf. ...	X				
Black locust, <i>Robinia pseudoacacia</i> L. ....					X
Hardy catalpa, <i>Catalpa speciosa</i> Warder				X	
Chinese elm, <i>Ulmus parvifolia</i> Jacq. ....				X	
Black walnut, <i>Juglans nigra</i> L. ....				X	
Little walnut, <i>Juglans rupestris</i> Engelm.				X	
Green ash, <i>Fraxinus pennsylvanica lanceo-</i> <i>lata</i> (Borkh.) Sarg. ....			X		
Ailanthus, <i>Ailanthus altissima</i> (Mill.) Swingle ....				X	
Pecan, <i>Hicoria pecan</i> (Marsh.) Britt. ....			X		
Post oak, <i>Quercus stellata</i> Wang. ....				X	
<b>Conifers:</b>					
Austrian pine, <i>Pinus nigra austriaca</i> Schneid. ....				X	
Ponderosa pine, <i>Pinus ponderosa</i> Laws.				X	
Arizona cypress, <i>Cupressus arizonica</i> Greene ....				X	
Eastern red cedar, <i>Juniperus virgin-</i> <i>iana</i> L. ....		X			
Rocky Mountain red cedar, <i>Juniperus</i> <i>scopulorum</i> Sarg. ....		X			
Oriental arborvitae, <i>Thuja orientalis</i> L.			X		
<b>Tall shrubs:</b>					
Apricot, <i>Prunus</i> sp. ....				X	
Redbud, <i>Cercis canadensis</i> L. ....				X	
Western soapberry, <i>Sapindus drummondii</i> H. and A. ....					X
Gum elastic, <i>Bumelia lanuginosa</i> (Michx.) Pers. ....			X		
Texas pistache, <i>Pistacia texana</i> Swingle ...			X		
Russian-olive, <i>Elaeagnus angustifolia</i> L. ...				X	
Rough-leaf dogwood, <i>Cornus asperifolia</i> Michx. ....					X
<b>Low shrubs:</b>					
Desert willow, <i>Chilopsis linearis</i> (Cava- nilles de Candolle) ....			X		
Tamarisk, <i>Tamarix gallica</i> L. ....			X		
Lilac, <i>Syringa</i> sp. ....					X
Hawthorn, <i>Crataegus</i> sp. ....				X	
Chickasaw plum, <i>Prunus angustifolia</i> Marsh. ....				X	
Sumac, <i>Rhus</i> sp. ....					
Coralberry, <i>Symphoricarpos orbiculatus</i> Muench. ....	X				



through the soil. They are the sole agents responsible for the direct penetration and infection of the roots of susceptible plants. The mycelial weft prominent on the roots of diseased plants is so typical that the fungus can be readily identified with a hand lens, and only in few instances must a microscope be resorted to.

Second, the sclerotia, small, buff, compact bodies varying in size from that of a mustard seed to that of a wheat kernel, in shape from irregularly spherical to oval, and in numbers from several to many, are usually found beneath diseased plants at different levels in the soil. They are produced, in the main, in a bead-like arrangement, from a series of enlargements of the rhizomorphic strands. Like the strands, the sclerotia may remain dormant indefinitely under adverse conditions and resume growth when the proper environmental conditions are at hand.

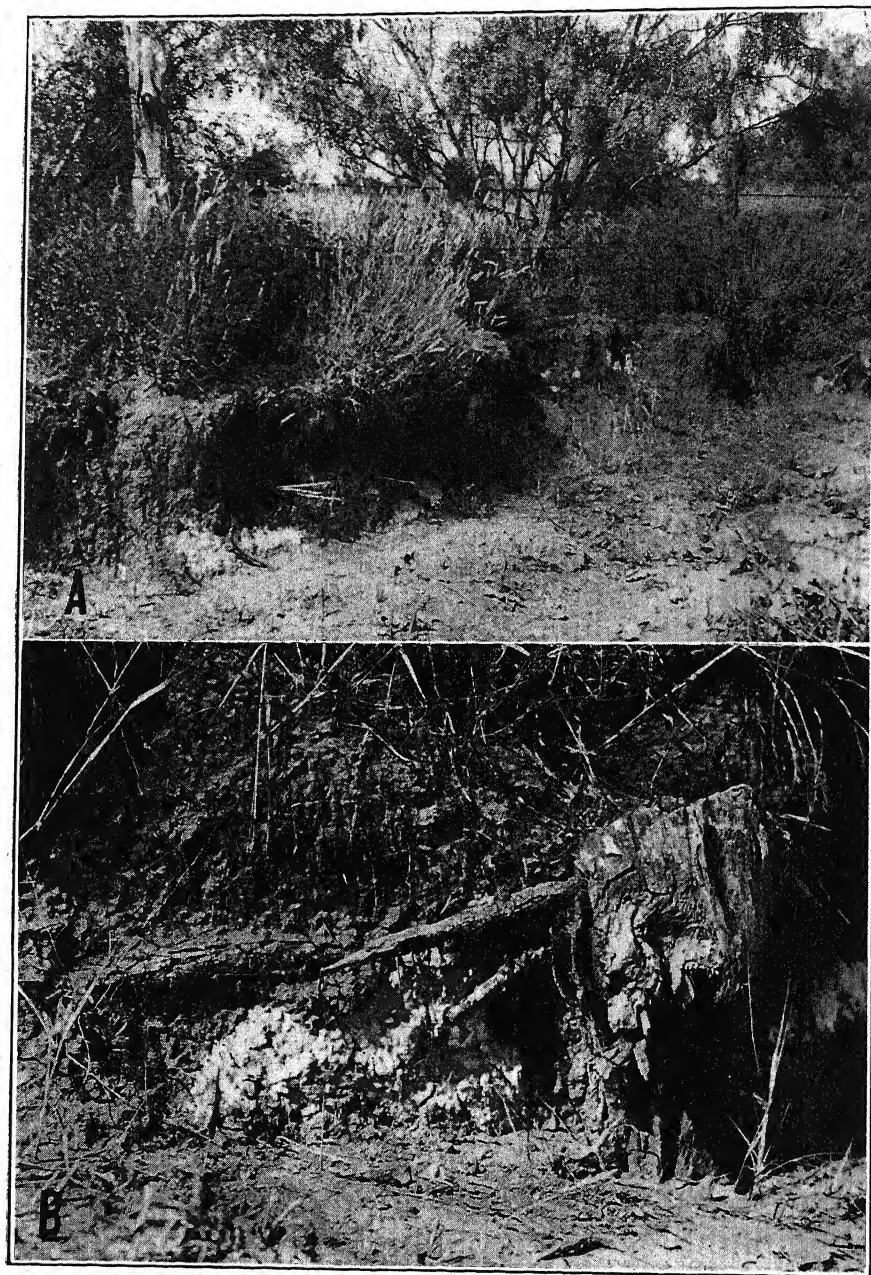
Third, the spore stage, (*Phymatotrichum omnivorum* (Shear) Duggar), which first appears on the surface of the soil in the form of a dense, fluffy white mat of mycelium. The size and thickness of the conidial mats vary with weather conditions. Depending on the duration of cloudy weather with rain, together with a high soil moisture and high relative humidity, the spore mats may become from 1 to 12 inches in diameter. Within 24 to 48 hours after first appearing, the creamy white mycelium is replaced by spores, which in the mass are of a buff color. These conidial mats may appear on root-rot-infested land, either cropped or virgin, at any time during the active growth of the fungus, but only when conditions for their formation are favorable. Although several investigators have obtained a low percentage of abortive germination, none has been able to produce mycelial growth from spores, nor to cause infection of any plant with spore suspensions. Thus, for the present, the conidial stage can be considered functionless.

#### SURVEY METHODS

Diseased or dead plants were carefully lifted from the soil by means of a tile spade and their roots were examined for the presence of *Ozonium* strands. If the roots of one or more plants growing on a square mile of land showed the typical mycelial weft, the entire section was, for the purpose of the preliminary survey, considered infested, even though root rot might then have been confined to a small area. Since the formation of sclerotia in the soil can be due only to the presence of *Ozonium* strands, a limited amount of soil sifting to find the sclerotia was attempted.

Owing to the higher than normal precipitation for the spring and summer months of 1935 in the Shelter-belt Zone, probably a nearly maximum amount of infection by *Ozonium* occurred on all types of plants and likewise an abundance of conidial mats were produced during June and July. The mats were found under various conditions in a number of counties in virgin and

## PLATE I



A.—A series of conidial mats along a road bank, extending for some distance. Weeds infected with *Ozonium* were present in the mesquite thicket and in the wash at the side of the road.

B.—Conidial mats growing in conjunction with the roots of a mesquite stump. (Haskell County.)

pasture lands and in fields devoted to many kinds of crops (Plate I, A and B). Here again the presence of conidial mats, in the absence of any susceptible plant indicators, on any square mile of land was thought sufficient to classify the section as an infested one.

The susceptible plants examined for *Ozonium* infestation in virgin and pasture lands consisted of the more common native herbaceous plants, shrubs, and trees, together with annual and perennial weeds growing along the roadside and, in many instances, in association with the above-named types of plants. On areas devoted to nonsusceptible crops, weeds were depended on to a great extent (Fig. 1). On lands cropped to susceptible

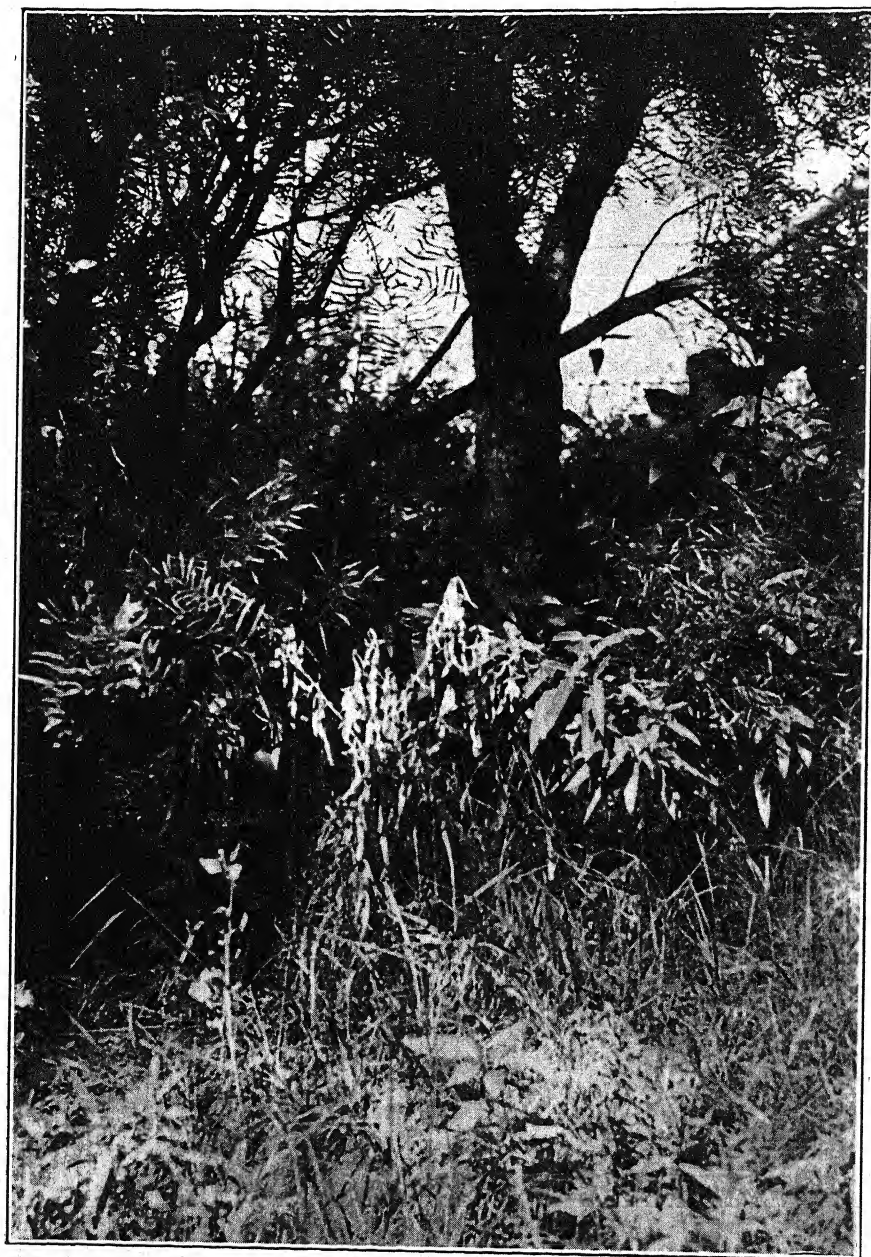


FIG. 1.—A diseased white horse nettle growing in a maize field, indicating that *Ozonium* is present in the soil. (Baylor County.)

plants, such as alfalfa and other legumes, the root-rot spots were more obvious. As cotton was late in this section, root rot was not observed in cotton fields until the first week in July, so that, up to that time, susceptible weeds had to be used as indicators of *Ozonium* on land devoted to cotton. Garden and truck patches, farm orchards, roadside, city, and farmstead plantings of trees and ornamentals were also checked for diseased and dead plants and the roots examined for the presence or absence of *Ozonium* strands.

For the most part the greatest dependence was placed on 3 very susceptible weed indicators, present throughout the Shelter-belt Zone, *i.e.*, the nightshades or horse nettles, particularly the white horse nettle, *Solanum*

## PLATE II



A typical roadside view of diseased white horse nettle, at the base of the ubiquitous mesquite. A recently cleared field planted to cotton lies beyond the fence. (Haskell County.)

*eleagnifolium*, the ragweeds, *Ambrosia* spp., and lambs' quarters, *Chenopodium album*. The universal distribution of these 3 weeds in native pasture, cropped, and waste lands, along railroad embankments, roadsides, ditches, washes, on the banks of small dry creeks, and on the overflow areas of the larger streams, aided very materially in delimiting the ozonium-infested areas from the noninfested areas (Pl. II). The cockleburs, *Xanthium* spp., ground cherries, *Physalis* spp., sunflowers, *Helianthus* spp., and thistles, *Cirsium* spp., together with other less common annual and perennial weeds, also served as susceptible indicators in the root-rot survey.

The general procedure in mapping the root-rot-infested areas was briefly as follows: An idea of soil types, location of agricultural lands, contours, and drainage systems, including dry washes, creeks, and larger streams, was obtained from available maps and through consultation with county agricultural agents. All available information regarding relative prevalence of root rot also was obtained from them. On the basis of this information, the county was divided into several districts and a reconnaissance of each district was made. When root rot was first found, either on plants or in the form of conidial mats, an intensive section-by-section survey was conducted until the entire area of root-rot infestation was determined. This procedure was followed in the areas under cultivation, since lack of roads in certain non-agricultural areas precluded anything more than a reconnaissance. Continuity of infested sections, and relative prevalence of *Ozonium* were determined by the presence of diseased or dying host plants or conidial mats and by the number of host plants attacked by the fungus.

#### RESULTS OF SURVEY

The distribution and degree of infestation of ozonium root rot, as determined by the survey, is presented in figure 2. During the progress of the survey, and again when the map was being prepared from the data obtained, the writer was impressed with the apparent relationship between root-rot infested areas and the water-sheds and drainage basins of the rivers that have either their source in, or run through, the Shelter-belt Zone. For simplicity, the results of the survey will be discussed on the basis of water-sheds and drainage basins of the larger streams. As no root rot was found in the four northern counties included in the Shelter-belt Zone in Texas, they have been omitted from the figure. A reconnaissance opposite these counties in Oklahoma showed absence of root rot in the drainage areas of the two forks of the Red River and beyond their junction for a distance of 20 miles, with the exception of a localized area about 5 miles along a small tributary in the northwestern part of Tillman County.

In Texas only two isolated spots of root-rot infestation were located in the drainage basin of the Prairie Dog Town Fork. The first consisted of

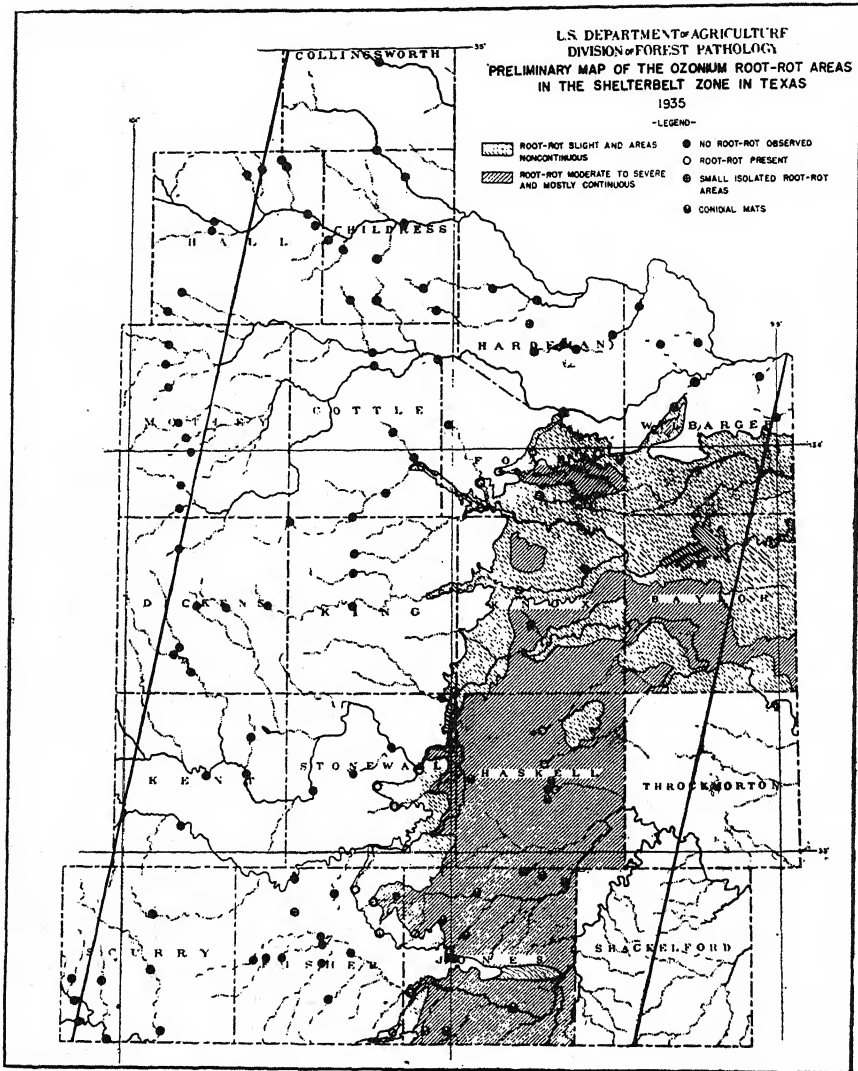


FIG. 2. The distribution and prevalence of ozonium root rot in the Shelter-belt Zone of Texas.

an area equaling four city blocks in the town of Memphis, Hall County, and the second area, approximately three sections in extent, lies three miles west of Quanah, Hardeman County. Since root rot was not found elsewhere in either county, it may be assumed that *Ozonium* was introduced into these limited areas on infested nursery stock, where it has persisted and gradually spread from one or more foci in the course of years.



The Pease River and the majority of its tributaries have their origin in the western part of the Shelter-belt Zone; this drainage basin covers the greater part of several counties and finally empties into the Red River in northeastern Wilbarger County. Root rot was found at only two points in this large drainage area, in the valley of Raggedy Creek in Foard County and Paradise Creek in eastern Foard and Wilbarger Counties. In the first instance, root rot was found generally distributed throughout the narrow valley consisting mostly of virgin land. Root rot was observed in the larger valley of Paradise Creek attacking a number of field-crop plants, fruit and shade trees and ornamental shrubs, as well as weeds. Conidial mats also were plentiful in late June in alfalfa fields. Both valleys are delimited by sand ridges which restricted the distribution of *Ozonium*. Apparently root-rot infested areas in the Pease River basin are strictly confined to the valleys of Raggedy and Paradise Creeks and in both instances are sharply delimited by sand ridges.

The entire water-shed of the Wichita River lies within the Shelter-belt Zone and a large part of the area is drained by this river. The most western locations of *Ozonium*-infested land in this basin were found in southeastern Cottle County at a point where the North Fork contains running water for the greater part of the year, in southwestern Foard County on the Middle Fork beyond its junction with the North Fork, and in the valley of the South Fork in eastern King County. Observations beyond these points showed root rot absent.

*Ozonium* root-rot was noted throughout the area between the North and South Forks of the Wichita River and it was especially prevalent in the agricultural area about Truscott in Knox County. Here the extreme susceptibility of Chinese elms was observed in a young four-year-old grove, where 50 per cent of the trees were diseased, dying, or dead. In Foard County, root rot was distinctly pronounced throughout the course of Beaver Creek, a rather large tributary of the Wichita River.

Only a small portion of the water-shed of the Little Wichita River has its origin in the Shelter-belt Zone. Root rot was especially prevalent, mostly in virgin lands, throughout this drainage basin. Diseased weeds found along the highways and railroad embankments served as the only locations where susceptible plant indicators could be found. This was particularly true in large virgin and pasture areas of northern Baylor and southern Wilbarger Counties.

More than half of the Shelter-belt Zone lies in the drainage of the Brazos River, comprising three large forks, the Salt and Double Mountain whose water-sheds lie beyond the Zone and the Clear Fork draining all or a portion of four southern counties. The western limits of root-rot infested lands in the valleys of these forks were located in the eastern quarter of Stonewall and in the north and southeastern corners of Fisher Counties. The relative

distribution and prevalence of root rot in both virgin and cropped lands in the area between the three forks in these counties, is shown in figure 2. The disease was very prevalent in the entire region between the Wichita and Brazos Rivers in Knox and Baylor Counties and especially so in the better agricultural areas. Even on the divide between these two rivers, many diseased weeds were noted.

There was an area in northeast Haskell County consisting of rough and gravelly land where root rot was noted only occasionally. A noninfected area, made up mostly of sandy soil and probably the former bed of a stream, extends into central Jones County. A small persistent isolated area of ozonium-infested land was found directly west of Rotan in Fisher County, probably the result of introducing infected nursery stock.

Part of the water-shed of the Colorado River is in Scurry County. An intensive survey failed to locate any evidence whatsoever of Ozonium there, in spite of the abundance of susceptible plant indicators. Taubenhaus and Ezekiel (4) have reported root rot in both Scurry and Kent Counties, but the writer was unable to find any evidence of root rot in either county during the growing season of 1935.

On the basis of this preliminary survey the following counties in the Shelter-belt Zone can be safely considered ozonium-free; Lipscomb, Hemp-hill, Wheeler, Collingsworth, Childress, Motley, and Dickens. If we omit from consideration the 2 small isolated spots in Hall and Hardeman Counties we can include these counties. Although root rot was not observed in Kent and Scurry Counties, there remains the probability that small infested areas are present on the basis of the reports of Taubenhaus and Ezekiel.<sup>4</sup> The greater portions of the land area in Cottle, King, Stonewall, and Fisher Counties also are noninfested, although ozonium-infested areas have been found to varying extent in some of the eastern section of these counties. Most of the land in Jones and Knox and all in Haskell and Baylor Counties may be classified as harboring Ozonium. Approximately the southern half and the southern third of Foard and Wilbarger Counties can be placed in the same category. No intensive survey was made in Throckmorton and Shackelford Counties, since they consist almost entirely of native pasture lands, but enough evidence of ozonium root rot was seen to indicate that the fungus is generally distributed.

For lack of better landmarks, ozonium root rot in the Shelter-belt Zone may be said to be prevalent as far as 34° N. Latitude and approximately to 100° West Longitude. These limits, however, should be considered as tentative until they are rechecked.

#### DISCUSSION

The futility of opening native ozonium-infested land to the production of susceptible crop plants is strikingly evident, yet new tracts are being



cleared each year, not only in the Shelter-belt Zone, but in other areas of Texas and the Southwest as well. Through the opening up of large areas of virgin land to cultivation in newly irrigated sections of the Southwest, we may find that much of the value of such projects will be lost through ozonium root rot, so that, almost immediately, only certain nonsusceptible crops can be grown. It is futile, likewise, to plant trees susceptible to Ozonium, either in virgin or cultivated land infested by the fungus, since here again root rot will be one of the limiting factors in the final development of the Shelter-belt project in Texas. Therefore, it is expedient to avoid root-rot areas in the zone, or, if this be impractical, to plant only sufficiently tolerant or resistant trees so that the seedling mortality due to ozonium infection will be slight and a nearly normal growth will occur, in spite of the aggressiveness of the fungus.

Repeated observations indicate that when root rot is found at the headwaters of a stream it usually is distributed throughout the drainage basin, and its incidence increases at the lower levels. Prevalence and continuity of root rot from section to section also is more pronounced in the better agricultural than in the submarginal and virgin lands. This incidence of root rot is, perhaps, the result of an accumulation of the fungus in the better farming areas, due to the continued planting of highly susceptible crops, such as cotton, and the lack of suitable long-time rotations with nonsusceptible crops. By planting trees tolerant to Ozonium, it may be possible to reduce the losses to a minimum in the Shelter-belt plantings.

The writer already has stressed the sharpness with which noninfested areas are delimited from infested. Of the many seen during the survey, 2 illustrations of the condition will be mentioned. The headwaters of Thompson Creek in Jones County, Texas, are encompassed by rather high ridges. No root rot was found to the west and north of this valley beyond the ridges, but, in the valley, conidial mats were observed on the face of the road cuts of the slopes, diseased weeds were plentiful in the mesquite thickets and along the roadside, and diseased cotton plants were noted in the first cultivated field. Similarly, in Tillman County, Oklahoma, the headwaters of Deadman's Creek are located in a semibowl surrounded by high ridges. Here also no root rot was found west of the divide. In the basin, however, Ozonium was noted on the roots of weeds, and conidial mats were found in a shaded area of a roadside ditch. From this point on, down the valley, incidence of root rot increased and cotton was dying in rather large spots in the first cultivated fields. While Ozonium has been seen in all types of soil from blow sand to a heavy adobe, it is apparent that the moisture-holding capacity of a soil is a more important factor than the soil type. Perhaps sufficient moisture seeps into the low basins making up the headwaters of the creeks from the surrounding ridges to perpetuate the develop-

ment of the fungus indefinitely. In this connection the deficiency of soil moisture and possibly low temperatures may be 2 of the contributing factors that limit both the western and northern infestation in the Shelter-belt Zone in Texas and southern Oklahoma. From the enormous amount of soil and plant débris washed down the drainage basins in 1935 in this region, one can readily realize why incidence of root rot increases at the lower levels.

#### SUMMARY

In view of the insidious nature of the indigenous soil-inhabiting fungus, *Ozonium omnivorum*, the infested areas within the Shelter-belt Zone extending into southern Oklahoma and Texas in advance of planting were mapped with the object of avoiding these areas or employing resistant trees. The susceptible plants employed as indicators of infested lands consisted of the more common endemic herbaceous plants, shrubs, and trees, together with annual and perennial weeds and many crop plants. The universal distribution of 3 susceptible weeds (horse nettles, ragweeds, and lamb's quarters) in virgin, pasture, waste, and cultivated lands, along railroad embankments, roadsides, fence rows, ditches, washes, banks of dry creeks and on flood plains of larger streams, as well as the presence of conidial mats, aided very materially in detecting *Ozonium*. A section of land was deemed infested when one or more diseased plants revealed the characteristic mycelial weft on the roots, or when conidial mats were found. On the basis of the above procedure the approximate limits of root-rot infestation were found to be south of 34° N. Lat. and east of the 100th meridian. In the main, prevalence of root rot was much more pronounced in the better agricultural lands and in the valleys. Repeated observations indicate that when root rot is found in the headwaters of a stream it usually is distributed throughout the drainage basin, and the incidence of root rot increases at the lower levels. The sharpness with which the infested and noninfested areas were delimited was especially striking.

## SOME EFFECTS OF PLANT DISEASES ON VARIABILITY OF YIELDS

CARL HARTLEY AND ANNIE RATHBUN-GRAVATT

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Plant pathology has been applied with generally recognized success to improving the quantity and quality of plant products and decreasing the cost of production. Another objective, that of reducing the variability of yields, is believed to deserve somewhat more consideration than it has received. Yields of most crops vary unpredictably from year to year, causing troublesome fluctuations in supply and price, increasing the difficulties that beset the agencies wishing to regulate production, and interfering with the security of the individual producer. The variation is, of course, the result of the effects upon yield of numerous factors, which in themselves are variable. It is much influenced by such factors as drouth. The present paper attempts to consider only the contribution to yield variation made by plant parasites and the effect that disease-control measures may have on yield variation.

Two groups of diseases that differ decidedly in their effects on yield variation can be distinguished. The first includes those favored by conditions that weaken the host. These are likely to decrease yields most in years when the crops would be poor anyhow, and thus tend strongly to increase the variation in annual yields. The second includes those favored by conditions that are otherwise favorable to the host; these tend to pull down the production of the peak years, more than that of the poor years, and are, therefore, less likely to increase the yield variation; they may even decrease variation. There are, of course, intermediate diseases, which are as likely to be severe in good years as in poor years, and, therefore, have an effect on variation intermediate between the two groups. Further analysis will be facilitated by use of the customary measures of variation and correlation.

### VARIABILITY OF REGIONAL YIELDS AS AFFECTED BY A SINGLE DISEASE

The addition of any independently varying factor necessarily increases the variation in the end results. Fortunately, the variation due to an additional factor is not directly additive to the variation produced by other factors. If the standard deviation of the annual yields of a particular crop were 4 bushels per acre and a new disease appeared causing losses that had a standard deviation of 3 bushels, the standard deviation of yields after the establishment of the disease would be not the sum of 4 and 3, but the square root of the sum of their squares, or 5 bushels.

In actual fact, most diseases are not independently varying factors. Interrelations are the rule. It is scarcely possible to conceive of a disease whose progress is not influenced by the vigor of the host, or by environmental factors that also affect the yield in some other way. The dependence of the disease on conditions affecting the host may be expressed quantitatively in the coefficient of correlation between the disease loss and the yield that would be had in the absence of disease. If disease loss increased as the disease-free yield decreased, this coefficient of correlation would be negative and the effect of the disease in increasing yield variation would be accentuated. Thus, with a correlation  $r = -0.5$  between disease loss and the yield to be had without disease in the imaginary example in the preceding paragraph the variation in yield would have been increased by the new disease to 6.1 bushels instead of 5 bushels. A perfect negative correlation would have made the disease variation directly additive, thus bringing the standard deviation of the actual yields to the full 7 bushels. If, on the other hand, the disease losses increased under conditions otherwise favorable for high yields, the correlation between disease loss and yield in the absence of disease would be positive. This would result in a final variation less than that resulting from a disease that was independent of other yield factors, and might result even in a variation smaller than would have occurred in the absence of the disease. Thus, a correlation of  $+0.5$  in the example would result in a final standard deviation of 3.6 bushels, or less than the 4 bushels that represented the variation before the disease appeared, and a perfect positive correlation would have reduced the total variation to 1 bushel.<sup>1</sup>

To turn from synthetic to actual examples: The variability of the loss factor itself is shown for several important diseases by Faris, Stevens, and their associates (4, 8, 9, 11). The relative variation, and occasionally even the absolute variation, can be higher for the losses than for the yields themselves (Table 1).

Diseases that are negatively correlated with the yield that would have occurred in the absence of the disease, are probably most numerous among

<sup>1</sup> A more complete expression of the importance of correlation in determining the effect of a disease on the variation in annual yield is to be found in the usual formula for computing the variance of a difference. The actual yield is regarded as the difference between the disease-free yield and the estimated loss. The variance of the actual yield is, therefore, expressed by the formula  $\sigma_A^2 = \sigma_F^2 + \sigma_D^2 - 2r_{FD}\sigma_F\sigma_D$ , in which A is the actual yield, F the yield as it would be if free from the disease, and D the loss caused by the disease. From this it appears that the variation of the yields is always increased by the disease if  $r$  is negative; for it to be decreased by the disease,  $r$  must be not only positive, but it also must be large or  $\sigma_D$  must be small. For crop-planning purposes it is the coefficient of variation rather than the standard deviation that is important. This coefficient is more apt to be increased by disease than is the standard deviation because a disease pulls down the mean yield. A formula showing the effect of correlation on the relative magnitude of  $CV_A$  and  $CV_F$  would be complicated.

root rots and vascular diseases for which it is difficult to secure estimates of loss. Some indicative figures are available, however, in connection with fusarium wilt of cotton, which will serve to illustrate the previous generalization. This wilt is a disease for which liberal fertilization to produce more vigorous plants is recommended as one method of control (6), and in which negative correlation might, therefore, be expected. The yield per acre that should have been obtained in absence of wilt was computed for each year by adding the estimated loss to the reported yield. The correlation found between the losses and the hypothetical wilt-free yields for the United States as a whole, for the years 1920-1929 was  $-0.36$ ; these wilt-free yields have a coefficient of variation of 11.4 per cent; the reported actual yields show a variation of 12.0 per cent. The disease thus appears to have increased the yield variation. Its effect on variation was small because the variation in the estimated losses was small; but the effect on the standard deviation was 14 times as great and on the coefficient of variation nearly twice as great as it would have been in the absence of correlation. It is unfortunate that figures are not available for some negatively correlated disease that produces larger and more variable losses.

Among diseases that flourish with the same temperature, moisture, or other conditions favorable to the host, late blight of potatoes furnishes excellent illustrative material, and in this case the variation of loss is large

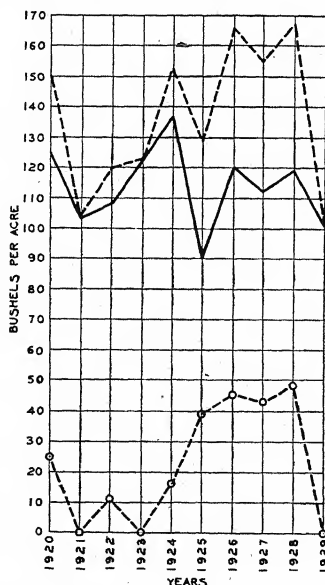


FIG. 1. Apparent effect of late blight on potato yields in New York. Solid line, reported yields. Lower broken line, estimated losses from late blight. Upper broken line, hypothetical blight-free yields.

enough to have a very considerable influence. The estimates for yield per acre in the State of New York for the 10 years studied (Fig. 1) have a standard deviation of 14 bushels. The annual loss estimates have a standard deviation of 20 bushels. But these variable losses, large at times, did not increase the variation in yield during these 10 years; in fact, if one assumes the approximate correctness of the yield and loss estimates, complete disease control would have made the annual yield even more erratic. In figure 1, this is shown by the upper broken line obtained by adding the estimated loss to the reported yield and thus representing hypothetical blight-free yields. It is evident from inspection of the graph that these hypothetical blight-free yields are more variable than the reported yields; their coefficient of variation is, in fact, one and a half times as great.<sup>2</sup>

The explanation is in the high positive correlation between the estimated losses and the hypothetical blight-free yields. The coefficient of correlation is +0.82. In other words, the disease appeared at its worst in those years when the crop would otherwise have been the largest; disease losses, therefore, depressed the high points in the yield curve more than they did the low points. Late blight thus seems to have been actually a stabilizing factor, so far as the total output of New York State during these 10 years was concerned. For 6 of these same years independent disease estimates are available also for Maine and Pennsylvania, the other two eastern surplus late-potato States. The coefficient of variation of the hypothetical blight-free combined yields for all three States during the 6 years was more than one and one half times that of the actual yields. Table 1 gives more complete data for all the States in which the disease was reported as important at some time during the 10-year period studied.

<sup>2</sup> For a disease that causes losses as large as does potato late blight, the principal source of error in the hypothetical blight-free yields, in their coefficient of variation, and in the coefficient of correlation between them and the disease losses, is probably in the inevitable errors of estimate of the losses. No information is available on the accuracy of the estimates, beyond the knowledge that they are made by experienced men as the result of observation on numerous and widely distributed fields. The effect of errors in the case under consideration is probably to increase both coefficients. The diseases-loss estimates, however, are positively correlated with the reported yields, as well as with the hypothetical, so there is little doubt that a positive correlation would be found between disease losses and correctly adjusted hypothetical yields. For brown rot of peach, bunt of wheat, and ear rots of corn, Stevens (9) and Stevens and Wood (11) compared the estimates compiled by the Plant Disease Survey with independent estimates obtained in very different ways, with such generally good agreements as to increase greatly the confidence in the Plant Disease Survey estimates on the visible diseases of aerial plant parts. Even where estimates of damage are entirely correct, it is possible, of course, that if the disease had been absent some other factor might have become important in limiting yield. For example, if control of a leaf spot resulted in a 15 per cent greater area of active leaf surface, a marginal soil-moisture supply might become submarginal.

TABLE 1.—*Variation in yields of potatoes and in estimated losses from late blight, 1920-1929*

State	Number of years represented	Standard deviation of			Coefficient of variation of			Correlation of yield with per cent loss from late blight	
		Reported yield	Estimated loss from late blight	Computed blight-free yields	Reported yield	Estimated loss from late blight	Computed blight-free yields	Reported yield	Computed blight-free yields
Wisconsin .....	10	Bu. 15	Bu. 9	Bu. 22	Per cent 15	Per cent 175	Per cent 20	.58	.82
Minnesota .....	10	15	3	17	16	246	17	.42	.58
West Virginia .....	10	15	12	20	15	174	19	.10	.69
New York .....	10	14	20	25	12	88	18	.05	.82
Oregon .....	10	13	3	14	12	171	13	.53	.66
Massachusetts .....	9	21	21	18	19	140	15	-.67	.35
Maryland .....	9	19	4	19	18	78	19	.41	.54
Ohio .....	9	14	5	16	15	183	17	.26	.46
Maine .....	8	44	3	43	18	104	16	-.45	.23
New Hampshire .....	8	18	13	21	15	120	16	-.34	.31
Pennsylvania .....	8	14	19	26	13	110	21	.24	.82
Tennessee .....	8	13	6	17	19	225	23	.50	.73
Connecticut .....	7	16	5	16	14	129	13	-.25	.09
Average .....	9	17.4 <sup>a</sup>	9.5 <sup>a</sup>	20.8 <sup>a</sup>	15.1 <sup>b</sup>	142.6 <sup>b</sup>	17.2 <sup>b</sup>	.13 <sup>c</sup>	.60 <sup>c</sup>

<sup>a</sup> Weighted arithmetic mean.<sup>b</sup> Weighted geometric mean.<sup>c</sup> Weighted mean through Fisher's  $z$  transformation.

On the purely theoretical basis given in footnote 1, it is evident that not only potato blight, but any disease of any crop will necessarily have a stabilizing effect on yields if the size and variability of the losses are not too large and disease prevalence is favored by those conditions that are otherwise favorable to high yield. The dependence of the variation effect of a plant disease on the way in which it is correlated is shown graphically in figure 2 for Fusarium on potatoes. For each of the 9 States in which the correlation

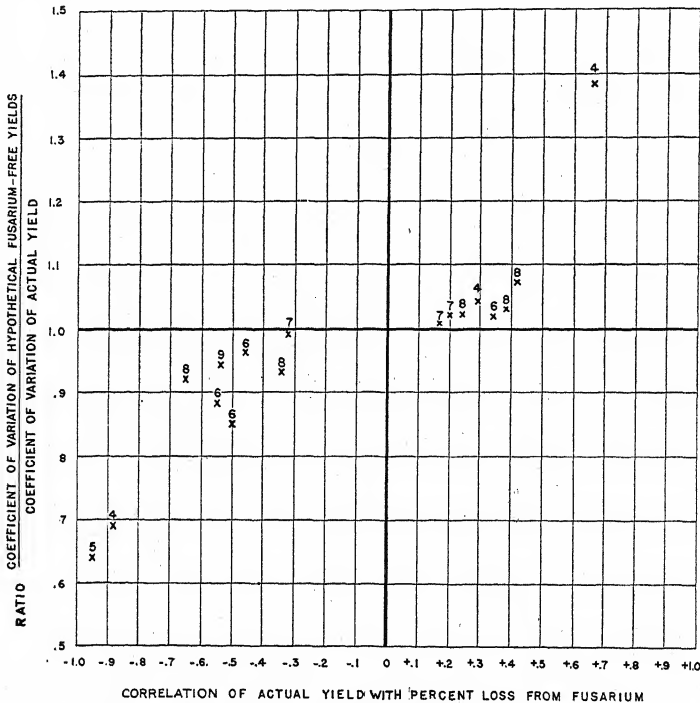


FIG. 2. Potato Fusarium data by individual States, illustrating the fact that the effect of a disease on yield variation depends on the correlation between loss and yield. The figure above each point shows the number of years for which data were available for the State concerned. The curvilinear relation is mainly due to the cramping of the correlation coefficients near the ends of the scale; the use of Fisher's  $z$  transformations in place of the correlation coefficients themselves would have shown a more nearly rectilinear relation.

was negative, the data indicate that the variation of Fusarium-free yields would have been less than that of the actual reported yields, while for each of the 8 States in which the correlation was positive, Fusarium-free yields would have been more variable. Were Fisher's  $z$  values used in place of the correlation coefficients, the relation would more nearly approach a straight line. Correlation of the loss with the disease-free yields in place of the actual would have resulted in moving the plotted points varying distances toward the right.



Even with a disease correlated positively with disease-free yield, like potato blight, the effect of the disease would be to increase yield variation if the loss variation were large enough. Figure 1 was based on average losses that occurred in fields most of which were sprayed. If regional figures were available for unsprayed fields the findings might be quite different. Before present control procedure was developed and with the varieties that were in use a century ago, the effect of late blight on national yields was anything but stabilizing. One has only to remember its part in causing the historic Irish famine (10) with its resultant loss of many thousands of lives and wholesale emigration to the United States. To put the case in another way, the progress that has been made in the control of the late blight has changed it from a catastrophic thing that upset all expectations, into one that no longer seriously affects the dependability of total regional yields so long as the rather expensive spraying schedules are followed. Not even the most extreme detractor of agricultural progress would be willing to return to potato-raising on the basis of 1845.

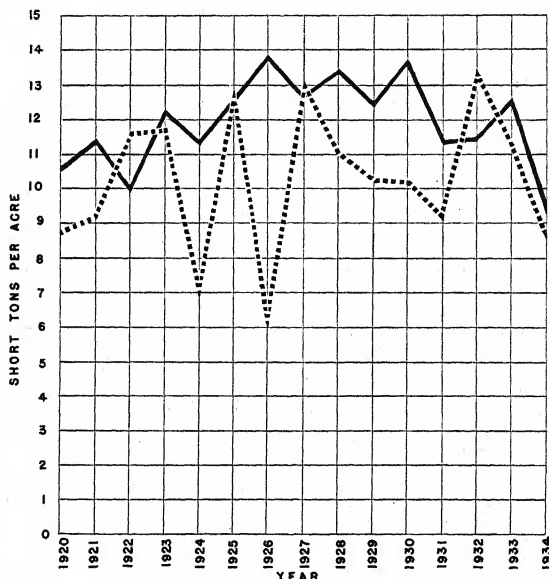


FIG. 3. Sugar-beet yields in Idaho and Colorado. Solid line, Colorado where 90 per cent of crop is grown outside the curly-top areas. Broken line, Idaho where curly top is prevalent.

Curly top of sugar beets furnishes a striking modern case of crop variation due to disease, irrespective of correlation. The effect can be examined by comparing yield dependability in Idaho and California where the disease is prevalent, against that in Colorado where about 90 per cent of the crop is grown outside the curly-top areas. Figure 3 shows the tremendous changes

in the reported Idaho production in the bad curly-top years as compared with the relative constancy of those for Colorado. For the entire 15-year series of yields<sup>3</sup> the coefficient of variation was 21 per cent in Idaho and only 11 per cent in Colorado. In the King City district in California, Carsner's figures (2) show yields to be even more erratic than those in Idaho as a result of the same disease. When the absolute variation in loss is so large, it must destroy the dependability of yield, no matter what the correlations may be. The curly-top-resistant varieties now coming into general use should result in much more stable yields in this crop. Recently, Brandes and Coons (1) called attention to the facts that yield fluctuations in certain States are definitely assignable to curly top and that sporadic low yields in certain other States are due to *Cercospora* leaf spot.

The foregoing discussion has been directed mainly to regional yields. Table 2 gives some similar data for national yields. Production totals for a crop grown generally over an area as large as the United States are, of course, less affected by disease fluctuations than are regional yields, because all parts of the area are not likely to suffer heavy losses in the same year. But for crops that, because of weight, bulk, or perishability, cannot be shipped economically from region to region, the effects of a regional epidemic can be nearly as serious to the consumers of that region as would a world shortage. On the other hand, such an epidemic in a crop that can be shipped long distances is more harmful to the producer than a world-wide epidemic, because he suffers reduction of output without profiting from a corresponding increase of price, as brought out in the enlightening discussion by Smith and associates (7). Regional effects of disease on variation are, therefore, in some respects more important than national. For certain crops the national production is so restricted in area that regional loss is national in effect; such a situation is analyzed by Stevens<sup>4</sup> for cranberry.

The ordinary measures of average variation have their greatest utility in connection with values that are determined by numerous factors of approximately equal importance and which, therefore, fall into approximately normal distributions. There are certain disease effects that depart too far from the ordinary to be adequately expressed by the usual variability measures. In view of the sudden geometric increases that sometimes occur in populations of insects, such occasional extreme results are perhaps more likely with a disease like curly top of sugar beets, in which the insect vector is the main controlling factor, than with diseases that are dependent on several factors of the same general order of magnitude. The single factor most likely to produce a completely unpredictable event in the phytopathological

<sup>3</sup> Yields taken from U. S. Department Agriculture Yearbooks 1924 to 1935.

<sup>4</sup> Stevens, N. E. An attempted analysis of the economic effects of cranberry diseases. U. S. Dept. Agr. Plant Disease Repr. 19: 112-128. 1935. [Mimeographed.]

field is the movement of a pathogen from one region or continent to another in which there is a hyper-susceptible host. Such cases are too well-known to require cataloguing.

#### VARIABILITY OF A CROP AS AFFECTED BY MORE THAN ONE DISEASE

The combined effect on yield variability of two or more diseases that attack a crop evidently depends not only on the amount of variation in the losses and their correlation with yield, but also on the extent to which the diseases are correlated with each other. If one of 2 diseases is favored by cold weather and the other by hot, the losses they cause are likely to be negatively correlated with each other, and their combined effect on the variability of the annual yield figures less than that of either alone. Tip burn and late blight appear to be negatively correlated with each other; for the 4 States for which data were available for the 10 years studied, the correlations between percentage of tipburn and percentage of late blight of potatoes were  $-0.25$ ,  $-0.32$ ,  $-0.48$ , and  $-0.62$ . On the other hand, if a crop be attacked by two diseases that are favored by the same conditions, as is reported by Folsom (5) for potato late blight and botrytis blight, the combined effect of the two diseases on yield variation must be greater than the effect of either alone. Another example of additive variability from two diseases is provided by scab and stem rust of wheat. In 10 series of comparisons, in the last decade in Minnesota and North Dakota, of the annual yields<sup>5</sup> of susceptible wheat varieties with varieties classified merely as resistant to stem rust, the mean coefficients of variation were approximately equal, the variation of the yield of the susceptible varieties being only 1.04 times that of the resistant ones. On the other hand, tests in three localities in Iowa<sup>6</sup> showed Progress wheat, resistant to scab as well as to rust, yielding more and being less variable from year to year than the susceptible Marquis. At one place over a 6-year period and at another over a 5-year period, the coefficients of variation for Marquis were 1.5 times as great as those for Progress; and at the third place over a 3-year period the coefficient was 1.2 times as great. The accumulation of such comparative data through a longer period would be highly desirable.

Another illustration of the effect of a combination of diseases on the yield variability is found in unpublished data on sugar-cane furnished by R. D. Rands. The coefficient of variation for annual total yields of this crop in Louisiana increased from 16 per cent during an 18-year period centering

<sup>5</sup> Data supplied by S. C. Salmon, Division of Cereal Crops and Diseases, Bureau of Plant Industry.

<sup>6</sup> Burnett, L. C. Small grains. Information from experiments in progress. Iowa Agr. Expt. Sta. Farm Crops and Soils Sect., Farm Crops Sub-sect. Leaflet FC, 2. 1932; 4. 1933; 6. 1934. [Mimeographed.]

in the 90's to 28 per cent in a period of similar length after the invasion of 3 new diseases, a change that he believes to be mainly due to these diseases.

The apparent effects of more than one disease on the variability of yields are given in table 2. It is worth noting that the diseases of oats apparently increase variability of national yields to an extent quite out of proportion to the reduction in average yield.

TABLE 2.—*Variability in estimated yields per acre of various crops in the United States from 1920-1929*

Crop	Disease	Reported yield		Computed disease-free yield	
		Average	Coefficient of variation	Average	Coefficient of variation
Cotton	Fusarium wilt	162 lbs.	12.0	167 lbs.	11.4
Cotton	All	162 lbs.	12.0	190 lbs.	11.7
Oats	All	29.8 bu.	11.9	32.3 bu.	10.5
Potatoes	All	111 bu.	8.5	141 bu.	9.9
Potatoes	Late blight	111 bu.	8.5	117 bu.	10.9

#### DISEASE UNCERTAINTIES AFFECTING THE INDIVIDUAL PRODUCER

The variations caused by diseases in the returns to the individual farmer are on the whole more serious from the standpoint of social security than the national or regional yield variations. Farming continues an individual venture, crowded with risks too large for the individual to carry readily. The effect of disease in decreasing crop predictability is much greater for the individual holding or for a local community than for the State or national yields already considered. A disease that has relatively little effect on yield totals may be periodically disastrous to single farms. This has been the case, for example, in recent years with cotton wilt, which appears (Table 2) to have little effect on national yield variation. Every experienced pathologist has seen so many extreme local losses to farmers that there is no need for citing examples to the readers of this journal. The growers of tree crops are subject to perhaps even greater uncertainty than the ordinary farmers, because of the long period of cumulative risk to which they are subject.

There is little quantitative information bearing on the effect of disease variability on individual or local yields. Some indication as to the influence of diseases on local variation can be secured from the results of disease-control experiments. This approach is not without difficulties. In interpretation it must be kept in mind that a treatment sometimes has other effects than disease control; and that if it be uncertain in its action, it may itself become an additional factor affecting variability. In the same way, a resis-

tant variety may increase variation unless it is dependable in other respects, as well as in resistance to the disease in question.

A treatment may, of course, be profitable without decreasing variability. Records of potato spraying in Pennsylvania are available for 1918-1930 that, according to Denniston and Hodgkiss (3), "show that spraying, when properly done, has been as equally profitable in non-blight years as in years when blight was present". The coefficient of variation computed from their data on yields in the sprayed fields was greater by one fifth than that for the unsprayed fields.

Examples of treatments that decreased variation are furnished by the writers' data on damping-off control in coniferous seedlings. At the Bessey Nursery of the U. S. Forest Service the sulphuric-acid soil treatment was applied to sowings of jack pine made at 12 different times during a period of several years. The coefficient of variation for yields per unit quantity of seed during the entire period was 74 per cent in the nontreated plots and 34 per cent in the treated. At the Fort Bayard Nursery the variation of 6 nontreated plots of ponderosa pine was 30 per cent and that of the 12 acid-treated 17 per cent. At the Monument Nursery the variation of 8 simultaneously sown nontreated plots of Engelmann spruce was 17 per cent and that of 10 aluminum sulphate-treated ones was only 7 per cent; for 11 simultaneously sown pairs of plots of Douglas fir at the same nursery the variation of the nontreated plots was 32 per cent and of those treated with aluminum sulphate 5 per cent. These are not specially selected cases; they represent the available experiments in this field in which replications were sufficiently numerous for variability analysis. Variation in yield of sowings at different times makes it difficult for the forest nurseryman to gauge his sowing operations to the needs of the field planting program. Variation of yields, either of different sowings or in different beds of the same sowing, hampers control of stand density and thus of quality of stock.

Variation due to disease in individual holdings can make trouble in the marketing as well as in the growing process. The uncertainties introduced into the distribution of fruits and vegetables by the fungi that attack them are too well known to require comment. A type of uncertainty less generally known, is that introduced into the purchase and utilization of timber lands by the variation in the amount of hidden decay already in the trunks. The best available quantitative data<sup>7</sup> are for Douglas fir, our largest single source of saw timber. Gross or apparent yield represents the yield that would have been obtained had there been no decay. For 23 plots below the age of 350 years, in different parts of the optimum range of this species and all on sites

<sup>7</sup> Data taken from tables 1 and 3 and figures 3 and 9 of U. S. Dept. Agr. Technical Bulletin 286 entitled "Decay and other losses in Douglas fir in western Oregon and Washington" by J. S. Boyce.

classified as of second quality, the expected gross yields per acre were obtained from curves of volume over age prepared by the original investigator. For each plot the difference was then determined between the actual gross yield and the expected, and expressed as a percentage of the expected value. These deviations from expectation averaged 20.2 per cent. The deviations of the yields of undecayed wood from the expected undecayed yields for those ages, obtained in the same way, were found to average 26.7 per cent. The root-mean-squares of the deviations, which are roughly equivalent to coefficients of variation around a moving average, are 23 per cent for the gross and 32 per cent for the net yields. In other words, the variation from the expected yield of sound wood has been much increased by the decay. In practice a prospective purchaser of timberland usually buys on the basis of an actual cruise of the area concerned, which gives him a more reliable expectation value for gross yield than does the generalized curve over age; and since the accuracy of cruising in estimating sound volumes is much less than in estimating gross volumes, the part played by decay in causing actual yields to depart from estimate is in fact probably greater in proportion than is indicated by the figures given. Unfortunately, cruise estimates for the study plots are not available. The pathologist has made a real contribution to industry in this case, by developing criteria by which hidden decay can be better estimated and the cruise estimates of sound timber can be made more reliable; this does not necessarily decrease the variation itself, but attains much the same end in that it decreases the variation from estimate which has proven one of the difficulties in the utilization of the timber of the Pacific Northwest.

#### CONCLUSIONS

Diseases in general may be expected to increase variation in production. The larger the variation of the disease losses or the more the disease losses tend to occur at times when other injurious factors also are active, the greater is the increase they produce in the variation of yields. Some diseases, however, are most serious in years otherwise favorable to the crop. Such diseases, if the loss variation is not too large, actually may decrease the variation in annual yields of a region as a whole. Late blight of potatoes appears to have become such a case—once a prime source of uncertainty, causing one of the great famines of history, and still a source of expense and worry to the individual farmer, it has been reduced to an apparently stabilizing factor, so far as regional and national yields are concerned, and so long as the recommended spraying schedules are maintained.

Stabilizing the yields of the individual grower is more essential from the standpoint of the producer than stabilizing regional yields. Disease losses to local communities or individual growers are more variable than regional

losses and diseases are, therefore, more likely to increase the variability of local yields than of regional yields.

In the choice of problems for investigative emphasis, it is suggested that when other considerations are equal, those diseases are particularly in need of attention, from the standpoint of both producer and consumer, in which variation is large or is negatively correlated with annual yields, so that better control will reduce yield variation.

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# CLADOSPORIUM LEAF BLOTCH OF PEONY

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Because of an increasing severity of peony leaf blotch in several Wisconsin nurseries, a comprehensive study of the disease and its control was begun in the fall of 1932. The vigor of infected plants was not noticeably diminished, but foliage discoloration of such plants frequently detracted from the appearance and value of the marketable product. The causal organism was described briefly and named *Cladosporium paeoniae* by Passerini in 1876 (5, 7). The disease has received little attention in this country during the past 2 decades other than brief reports and general descriptions<sup>2</sup> (1, 2, pp. 244-246), although it probably was an unimportant malady in the United States for some years prior to these reports.

Cladosporium leaf blotch has been reported from 19 States in this country as well as from the District of Columbia, Alaska, Canada, and numerous foreign countries. Of its occurrence in Europe, Whetzel (8) states, "... leaf blotch ... is to be observed in almost every peony planting today. ... ." These reports relate primarily to commercial plantings of peonies where the cumulative effects of the disease have become so important as to destroy the ornamental value of the foliage desired in late summer and autumn.

## DESCRIPTION OF THE DISEASE

Leaf blotch of peony is encountered only on aerial portions of numerous peony varieties, although leaf symptoms are the most conspicuous. Initial infection becomes manifest on the ventral surface of the leaf as small, circular or oval discolored areas, usually  $\frac{1}{2}$ -1 mm. in diameter. Infection spots spread slowly and reach a diameter of 2 to 3 mm. before they penetrate through the thickness of the leaf. They may be present in greater or lesser numbers (Fig. 1, A) per leaf, depending on severity of infection; and, as their growth continues, they may merge, giving the leaf an irregular, blotchy appearance. The extent of the infection is easily recognized by the slow advance of the dull, chestnut brown of the lower surface and the glossy, dark purple of the upper surface. This discoloration remains throughout the season as typical of the disease, and is not to be confused with a natural change in leaf pigmentation sometimes occurring toward the end of the grow-

<sup>1</sup> The writer is indebted to Dr. L. R. Jones, Professor of Plant Pathology, University of Wisconsin, who suggested this research and gave generously of his valuable advice and assistance throughout the investigation.

<sup>2</sup> New Jersey Agr. Expt. Sta. Dept. Plant Path. Diseases of peony. (I + S Nursery disease notes 1(6)). 1928. [Mimeographed.]



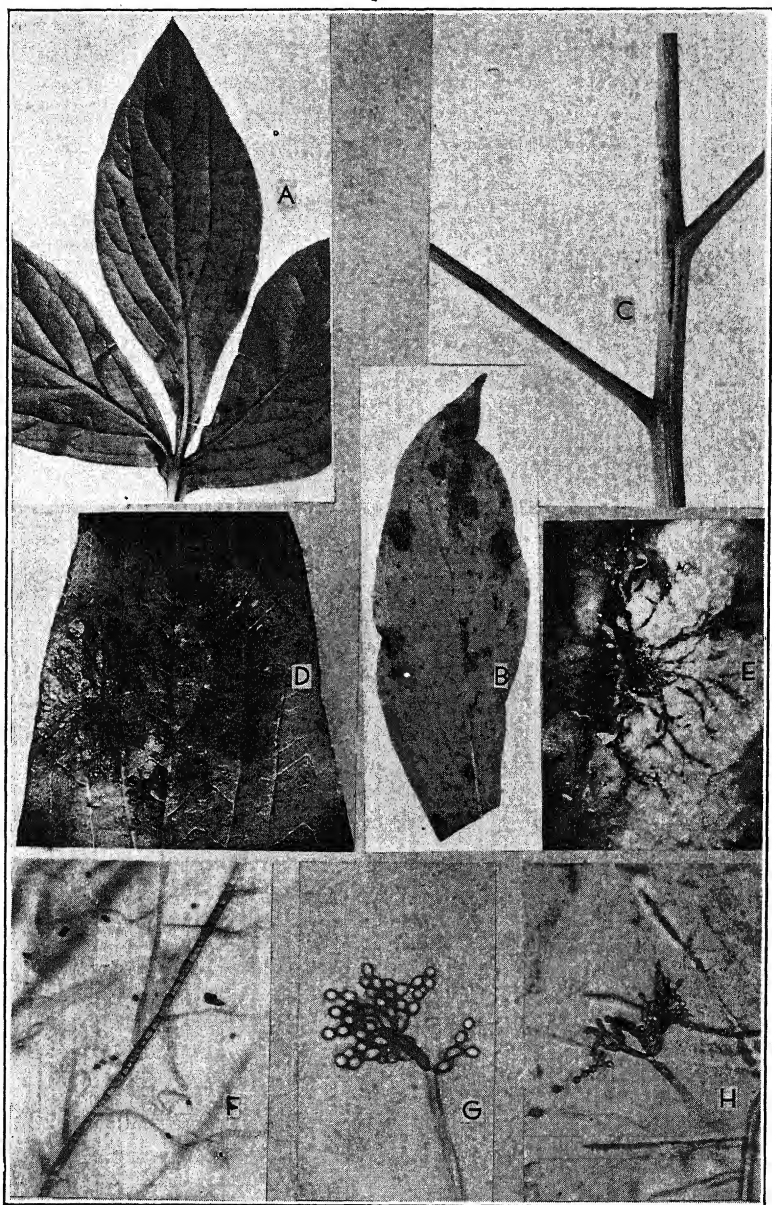


FIG. 1. *Cladosporium paeoniae* on peony foliage and in pure culture. A. Natural infection on the upper leaf surface. B. Dendriform growth of natural infection on the lower leaf surface when floated on 10 per cent sugar solution. C. Elongated lesions from natural stem infection. D-E. Artificial infection on excised leaflets. D. Slightly enlarged. E  $\times 7$ . F. Submerged mycelium and spores in potato-dextrose agar. Rounded cell contents atypical.  $\times 176$ . G-H. Mature sporophores and conidia on potato-dextrose agar. G  $\times 400$ ; H  $\times 240$ .

ing season. Unless abnormal drought conditions prevail, which may result in the collapse of the cells, the infected leaves retain their succulence and vigor throughout the season. Marginal infection predominates, especially near the leaf tip, and slight distortion may occur as growth continues. Outer leaves of the plant are ordinarily attacked first. The bushy nature of the host acts as a protection to the inner foliage against inoculum carried in spattering rain and wind.

Weiss<sup>3</sup> reports blossom infection, which he attributes to *Cladosporium paeoniae*, and Martin (3) reports the fungus on abortive buds. No infection of this type, however, has been observed in this region. The early blossoming period and short duration of the blooms appear to provide an escape from the slow-growing fungus, although simple leaves and leaf-like bracts located near the seed pods become severely infected, as well as the seed pods themselves. Petiole infection is relatively unimportant and is similar in appearance to stem infection.

On the young green stems, infection is first apparent as elongated, reddish brown streaks (Fig. 1, C) with slightly diffuse margins. The lesions present a plane surface, but, as growth continues, those near the crown of the plant are inclined to coalesce, darken, and become somewhat depressed. Lesions on the upper stems are similar in shape and color but are fewer in number. They tend to retain their individuality and become slightly raised rather than pitted. Infection spots are abundant at branch and petiole bases where débris of old infected parts and fallen petals may lodge and become a source of infection or provide favorable environment for the establishment of the fungus.

Any form of sporulation is conspicuously absent during the growing season, and only under favorable environmental conditions is the smooth surface of the leaf broken by masses of dark green conidia.

*Pathological Histology.*—In the early stages of leaf infection the slender hyphae of the fungus are found growing on the surface or partly or totally imbedded in the cuticle (Fig. 1, D-E). As the fungus develops, its surface ramifications become more abundant, but only seldom does a hypha penetrate a stomatal opening. Fresh material viewed in low magnification clearly showed the radiating hyphae in contact with discolored epidermal cells and leaf hairs, the pigmentation occurring several cells on either side of the superficial mycelium. This typical brown and purple discoloration always disappeared in prepared paraffin and cleared sections. Even those cells in close proximity to the mycelial threads appeared normal and healthy. No visible means of sustenance for the fungus were observed nor were any hyphae seen below the epidermal layers of the apparently healthy tissue. Slides of dead, infected leaf material collected in the field revealed abundant

<sup>3</sup> Weiss, F. Notes on some diseases of ornamentals. U. S. Dept. Agr. Bur. Plant Indus. Plant disease Repr. 16: 122-124. 1932. [Mimeographed.]

mycelium throughout the tissues and what appeared to be conidiophore stalks protruding through the stomata. There is the possibility that such conditions were due to secondary organisms or saphrophytes. *Cladosporium herbarum* was frequently isolated from collected material, and its appearance might easily be confused with that of the true pathogen. There is also the possibility that *C. paeoniae* is only slightly parasitic and can attack the inner tissues only after their death. Opportunity did not permit a more thorough investigation of this phase of study.

*Taxonomy*.—Passerini at Parma City, and Gorizia, Italy, first described the peony leaf-blotch fungus, naming it *Cladosporium paeoniae*. His very brief description of the fungus as found on *Paeonia edulis* was published in *Just's Jahresbericht* in 1876 (5). Saccardo (7, p. 362), besides publishing this description, but naming the host *P. officinale*, also gives Passerini credit for having discovered a variety of this organism, named *C. paeoniae anomala*, on leaves of *P. anomala* in the subalpine forests of Siberia. The existence of this new variety evidently is based on its occurrence on a different species of peony, but the two fungi probably are identical. No true identifying characters were given in these descriptions that might differentiate *C. paeoniae* from other *Cladosporium* species beyond the fact that it was pathogenic on peonies. Some difficulty was experienced in identifying the parasite because of this, for the study was begun late in the fall when natural foliage was no longer available, and it was only after forced peony foliage had been secured in midwinter that the authenticity of the fungus could be established by means of infection trials in the laboratory.

Only the conidial stage is known, which places it in the *Fungi Imperfecti* in the order Moniliales (Hyphales). Infected material, permitted to overwinter on the ground in net bags, was examined, without success, for spore forms other than conidia. Small masses of mycelium have been observed rounded up in very old agar tube cultures, but it is questionable as to whether or not these are abortive attempts to form sclerotia or ascigerous fruits. Numerous transfers were made of this type of mycelium in an effort to force these bodies to maturity, but without success.

*Morphology*.—In culture a great deal of variation may be observed in mycelial growth. Young, thin-wall hyphae usually are branched and composed of long, hyaline cells. With further development, the cell diameters increase and the walls become thickened and darker (Fig. 1, F). Typical chlamydospore formation has been observed in which excessively thick walls give the cells a rounded appearance.

In nature the conidiophores occur in erect, compact tufts and are usually of greater length than those observed in culture (Fig. 1, G-H). The frequently branched, septate stalks may vary somewhat with conditions, but, normally, their dimensions are similar to the hyphae from which they arise. Each sporophore may bear several branched or branchless chains of acrog-

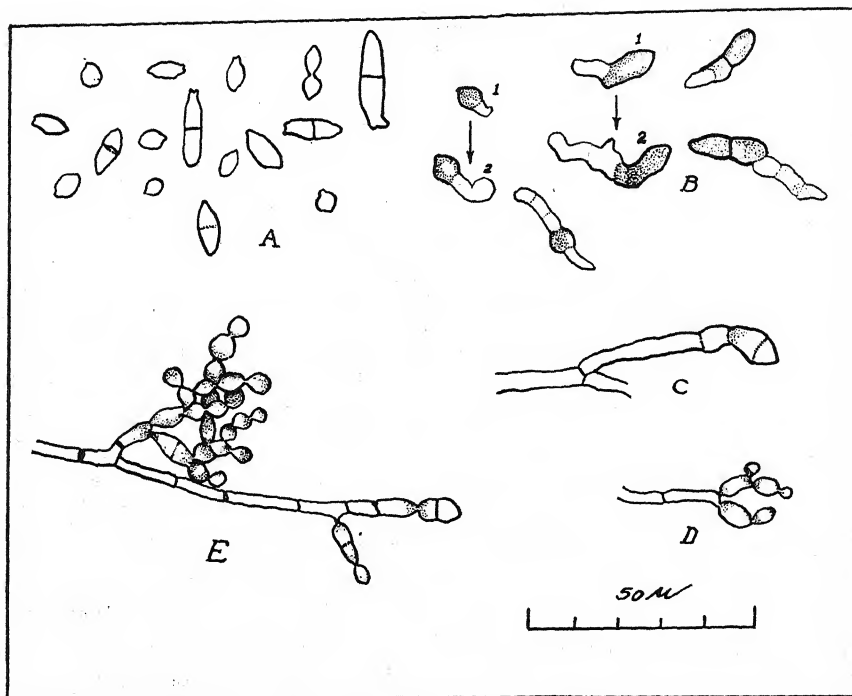


FIG. 2. *Cladosporium paeoniae* on malt agar. A. Variations in size and shape of conidia. B. Germination of conidia (1) at the end of 24 hours and (2) at the end of 48 hours. C.-D. Development of young sporophores. E. Mature sporophore.

enously formed conidia. The majority of the spores (Fig. 2, A) are small, round or lemon-shape and constitute the greater part of the branching chains. The larger ellipsoid spores may be either continuous or one-septate. These conidia are relatively few in number and represent the basal spores to which one or more small chains are attached. The place of attachment of detached spores is often marked by small geniculations at the ends of the conidia. Measurements of spores from malt agar tube cultures averaged  $6.4$  by  $3.7\ \mu$  for the small, round conidia,  $11.9$  by  $4.0\ \mu$  for the continuous ellipsoid conidia, and  $16.5$  by  $5.4\ \mu$  for the larger septate ellipsoid spores.

*Cultural Studies.*—First attempts to isolate the causal organism by the poured-plate method were hampered by the close association of numerous, fast-growing fungi. The slow-growing *Cladosporium paeoniae* could not compete with the faster growing secondary species of *Septoria*, *Penicillium*, *Rhizopus*, and a saprophytic *C. herbarum* that were often present. The true pathogen was easily isolated when it was discovered that successful sporulation could be obtained by placing dried infected material and 1-year-old pressed specimens in moist chambers for 5 days.

Single-spore cultures from all types of conidia from various plant parts

showed no marked differences in artificial culture. All cultural work herein reported is based upon single-spore cultures of the fungus.

The organism germinated and grew well on several liquid and solid media. Colonies growing on malt-agar plates increased their radial growth by an average of 2.4 millimeters per day at the optimum temperature. The color of young transfers for the first 3 days is Lincoln green (6). The color gradually changes and by the 18th day becomes an olive brown shade. The surface mycelium forms a fluffy covering over the agar with an abundance of conidiophores and conidia. Submerged mycelium appears beneath the surface of the agar at a point below the oldest portion of the culture in 10 days. The agar subsequently becomes thoroughly ramified by the subsurface development. *Cladosporium paeoniae* on malt-agar slants, sealed with lead foil, were kept alive and viable more than a year. At this age they had lost their pathogenicity.

Excellent germination was obtained in yeast-infusion glucose and 10 per cent peony decoction. Germination was slightly reduced in tap water and only about 15 per cent germination occurred in distilled water. Early stages of germination have been observed within 6 to 8 hours among all forms of conidia, while the majority of spores germinate in about 12 hours. It is not uncommon for 2 germ tubes to be borne from the ends of a single spore. In distilled water and peony leaf decoction, abnormal germination often occurred (Fig. 3, C, G, E) in which sporophores were formed directly from the spores soon after germination. On agar, germination was slightly slower than in liquid media, but growth appeared to be more normal. The vigor of the young germ tube during the first 20 hours appeared to be proportionate to the size of the spore or cell from which it emerged (Fig. 2, B).

*Pathogenicity.*—Time did not permit pathogenicity tests to be made in the field. Controlled tests, however, were made on forced peony foliage in the greenhouse and with excised leaves in the laboratory.

Healthy peony roots, dug in late fall, were subjected to a temperature several degrees below freezing from 18 hours to 12 days and "heeled in" the root cellar until needed. When potted, these plants produced normal, healthy young leaves in approximately 1½ months, and a few weeks later several plants were in full bloom. Many of the buds were abortive, and in some cases the buds were removed to stimulate more vigorous foliage growth.

Plants sprayed with a mono-conidial suspension developed leaf lesions in 16 days and elongated stem lesions were observed several days later. The spread of the organism was slow and dendriform (Fig. 1, D-E) similar to the type of growth produced on naturally infected plants when placed in a moist chamber (Fig. 1, B). Corresponding tests of pathogenicity, using the same methods of inoculation, were made on excised leaves floating on a 10 per cent sugar solution in Petri dishes (9). Leaves from various portions of the plants, both upper and lower surfaces, showed macroscopic lesions in 9 days. Subsequent growth was typically dendritic (Fig. 1, D-E). The

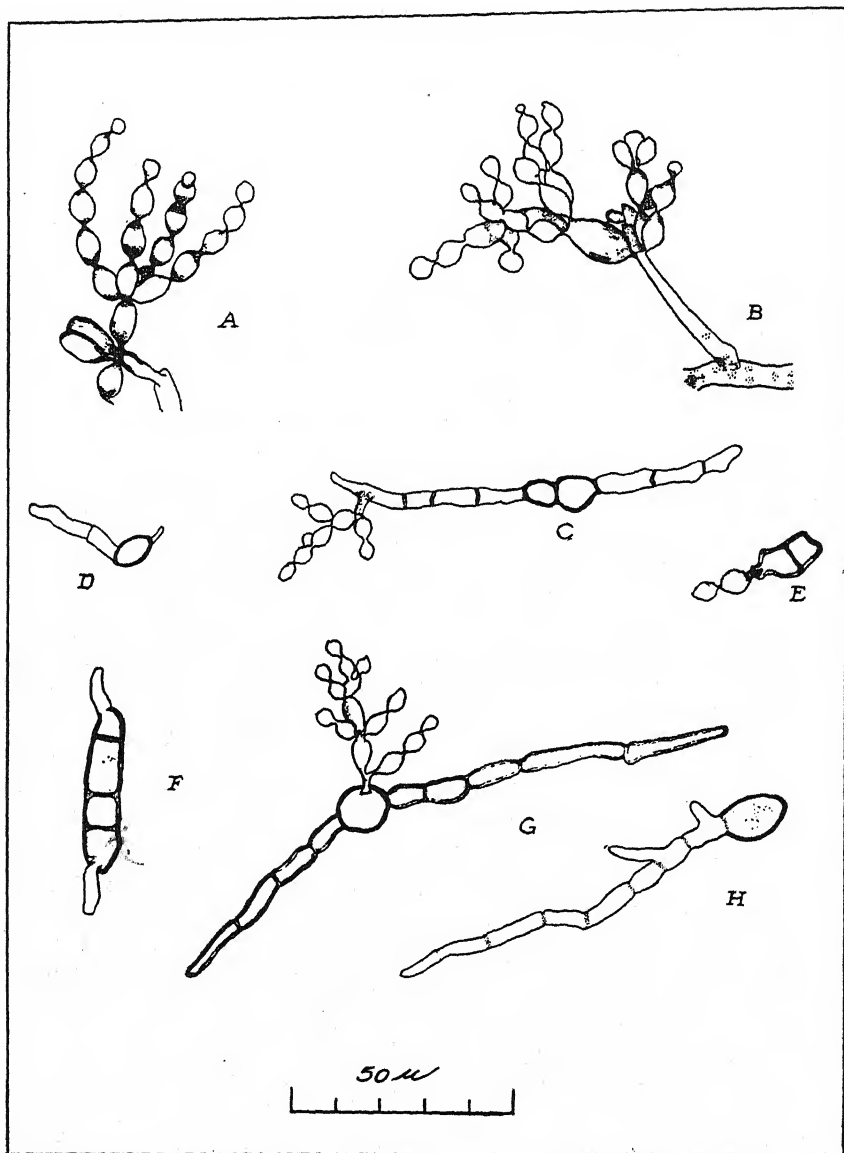


FIG. 3. *Cladosporium pasoniae* from hanging drop cultures, age 30 days. A-B. Mature sporophores showing typical chain formation of the conidia. Conidia in A from a single large basal spore. Yeast infusion glucose. C-G. C and E and G. Abnormal sporulation of germinating spores. C and G. In peony leaf decoction. E. In distilled water. D and H. Normal germinating spores in yeast-infusion glucose. F. Abnormally large, several-septate spore in the process of germination in yeast-infusion glucose.

reisolated organism, which was constantly associated with the foliage lesions, compared exactly with the microscopic and cultural characters of the original *Cladosporium paeoniae* inoculum. Reinoculation always resulted in the disease.

*Seasonal Development of the Disease.*—The first leaf infection of the season became evident shortly prior to the time of blossoming, or about June 1. The advance of the fungus throughout the season was slow, and from 5 to 90 per cent of the leaf area became discolored. Twig and petiole infection usually appeared several days later than leaf infection and was much slower in its advance. In many cases of slight infection stem lesions were absent or very inconspicuous; with more severe infection the number of stem lesions increased proportionately. Stem infection in no way appeared to effect the vitality or strength of the plants even though the stems were completely girdled with lesions as the season progressed.

Under natural conditions conidia were formed only in the presence of favorable moisture conditions provided by the late fall and spring rains. When such conditions were duplicated in moist chambers, successful sporulation was secured from dead, infected material. Tests were made in an attempt to force sporulation of living infected tissue by means of high relative humidity of the surrounding air. When these tests were made in July, there were no signs of sporulation in the field. The cut ends of similar, typically infected stems were sealed in flasks containing a 10 per cent sugar solution. The stems were then placed under 3 bell-jars, each jar being subjected to a different relative humidity. These controlled conditions were obtained by bubbling saturated air through solutions capable of reducing the atmospheric moisture to 50, 70, and 90 per cent relative humidity (4, p. 67-68). These figures may have been slightly higher due to the fact that transpiration water was not entirely expelled from the bell jars. Abundant sporulation was observed in 6 days on leaf lesions exposed to 70 and 90 per cent relative humidity. At the end of 9 days only very slight sporulation was observed on leaves in the 50 per cent chamber. A series of control stems exposed to the natural prevailing humidity showed no conidial formation. This experiment was repeated using tap water in place of the sugar solution with the same results.

Sporulation may be observed occasionally in late fall during precipitation periods. Tests were made to determine whether or not these spores could overwinter in the ground. Surface soil was collected near infected plants in the early spring, mixed with water, and the supernatant liquid used as inoculum. Microscopic examination of this solution revealed an occasional *Cladosporium*-like spore, but healthy peony plants used as a differentiating host remained noninfected when sprayed with the solution. That the mycelium overwinters in a dormant stage has been confirmed by masses of dark green conidia produced from overwintered material and even from pressed specimens over a year old. It is apparent that primary infection



originates from this overwintered material. No secondary infection occurs in this region, for no sporulation takes place on the current year's lesions during the growing season.

Observations in the field and laboratory indicated that conidia were disseminated primarily by meteoric water. It was apparent from examination of slides that conidia became detached promptly when in contact with water. Complete sporophores with attached chains could be examined only as they grew in hanging drops or on thin agar plates. Sporulating tissue could be shaken quite sharply in a dry atmosphere without detaching the spores. Attracted by the sticky sweet exudate on the buds, ants are frequently observed associated with the host at the time of infection. One may surmise that spores could be easily carried on and indirectly disseminated from the sticky appendages of these insects. Infection was noticeably severe on those portions of the plant most frequently traversed by the ants: namely, the bud bracts and surrounding single leaves. Some infection may be attributed to sucking insects, for small projecting tips, suspiciously similar to insect wounds, have been observed centrally located in very young lesions.

*Control Measures.*—Investigation of leaf-blotch control was undertaken at Turville's Nursery, Madison, Wisconsin, where an extensive planting of approximately 6 acres of severely infected peonies was made available for the study. The disease was present only on the broad-leaf, herbaceous varieties. The slender-leaf *P. tenuifolia* remained completely resistant in the midst of severe infection. This is of little consequence, for shortly after its early blooming period the entire plant normally dies down until the following spring.

There is a wide range of susceptibility among the broad-leaf varieties. Among several commercial plantings in which leaf blotch was prevalent, the most susceptible varieties were Oshkosh White, Felix Crousse, and Livingstone. Less infection appeared on Augustin d'Hour and Mathilde de Roseneck, still less on Louis Van Houtte, Edulis Superba, and Jules Calot, while Gigantea and Humei Carnea seemed quite resistant. As time is a significant factor in peony breeding and blooms of commercial value must be secured, breeding for resistance to leaf blotch becomes impracticable, especially when the disease may be controlled by other measures.

*Sanitation.*—In early autumn badly infected foliage was removed from 10 centrally located rows of plants. These were flanked on either side by plants whose foliage was allowed to remain as controls throughout the following growing season. Cut stems were placed in small piles throughout the field and burned. Additional sanitation involved burning thick layers of marsh hay on 2 plots 40 square feet in area and separated from each other by an unburned control strip. It was believed that such treatment would reduce still more the overwintering of infested material.

Observations the following summer indicated that uncut, unburned, flanking rows suffered more leaf blotch than the portion that had been sub-



jected to sanitation. The additional burning of marsh hay had further eliminated some of the disease. These results were determined by a comparison of susceptible varieties found in the different treated areas. It was concluded that burning tops in the autumn was a useful and commendable practice that would probably give excellent control if carried out from year to year. Excessive burning, however, by means of straw or hay does not produce a sufficiently greater increase in control over ordinary sanitation to warrant the expense.

*Sprays.*—Three different sprays were applied to infested areas chosen at random over the field, leaving suitable control strips between the treated plots. Two lime-sulphur sprays were applied to the soil, one in the late fall and the other in early spring. In late spring the third application of a 3-2-50 and a 1½-1-50 Bordeaux mixture was distributed evenly over clean, young plants on adjacent plots. All sprays were applied at the rate of 25 liters per acre.

The soil sprays did not appear to successfully reduce leaf-blotch infection. It was unfortunate that no highly susceptible variety was present in these sprayed areas to use as index plants. Both foliage sprays reduced blotching on some of the more susceptible varieties with the stronger mixture recommended as the better of the two. There is the possibility that the fungus had already established itself at the time of spraying, for 4 days later small lesions could be detected on the leaves. An earlier spray application might prove more beneficial.

*Transplanting.*—Three entire peony plants, identified by the nurseryman as belonging to the variety Louis Van Houtte, were removed from the infested field. The following day these plants were placed in another field far removed from the nursery and any known source of peony leaf-blotch infection. The first plant was handled with a minimum amount of disturbance of the soil, old stems, and the current year's foliage. This restored the plant to conditions similar to those under which it previously existed. The second plant was cleaned of as much soil as could be conveniently shaken from the roots, and the old tops were removed. The third plant was washed thoroughly in tap water and the old stems removed, as would ordinarily be done in careful nursery practice.

Early summer of the following year revealed easily visible spots on the first plant, although they were fewer and less advanced than those in the original, infested area. The plant from which the soil had been shaken had very little leaf spotting and only an occasional stem and petiole infection. The plant whose roots had been washed was nearly free of the disease. These observations were substantiated by another examination in the fall.

Parallel experiments in which the peony roots were treated with various concentrations of formaldehyde, Semesan, and Corona produced results comparable to those secured when the roots were washed in water.

It was concluded that transplanting, even with old debris present, re-

duces foliage infection, while the differences between washed and unwashed roots were so slight as to be considered insignificant; both practices are to be highly recommended in reducing the amount of infection. It seems likely that the disease might easily be controlled by moving an infested planting to another portion of the nursery and by taking precaution to remove the soil and infected tops as would ordinarily be done before marketing the stock. The feasibility of such a step has been observed in a consignment of approximately 50 plants acquired from the infested area by an adjoining park. The following spring new foliage remained disease-free, although the nursery was only 300 yards distant. The short range of the disease is noticeable in this example, and the dependence of the fungus upon meteoric water as a means of dissemination also probably accounts for the slow yearly increase of the disease in a neglected planting.

#### SUMMARY

A detailed description of peony leaf blotch, as caused by *Cladosporium paeoniae* Pass., together with some cultural, physiological, and pathological studies, are offered in an attempt to facilitate a clearer understanding of the disease.

The economic importance of peony leaf blotch is confined primarily to large peony plantings, and its effect is cumulative from year to year unless proper steps are taken to eliminate it.

Results of control experiments indicate that careful sanitation and destruction of old foliage each year or transplanting clean roots to non-infested areas or a combination of the two are recommended practices to eliminate the disease.

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# A STUDY OF THE REACTION OF F<sub>1</sub> OAT HYBRIDS AND THEIR RESPECTIVE PARENTAL LINES TO INOCULATION WITH SMUTS AND RUSTS

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Oats are difficult to hybridize, and usually only a few crossed seeds are obtained. Investigators, therefore, have refrained from inoculating such seed with disease organisms for fear of destroying the F<sub>1</sub> plants after much time and labor had been expended in effecting the desired hybrids. Few, if any, reports are available of attempts to inoculate and study simultaneously the reaction of F<sub>1</sub> oat plants to more than one of the major diseases of oats. Several reports, however, are known that relate to studies of resistance to rusts.<sup>2,3</sup>

## EXPERIMENTAL PROCEDURE

In the summers of 1934 and 1935, numerous oat crosses were made by the junior writer at the Aberdeen, Idaho, Substation. One or both of the parents of nearly all crosses were resistant either to stem rust (*Puccinia graminis avenae* Eriks. and Henn.) or to crown rust (*P. coronata avenae* F. and L.) or to the smuts<sup>4,5</sup> (*Ustilago levis* (Kell. and Sw.) Magn. and U. *avenae* (Pers.) Jens.). The F<sub>1</sub> seeds obtained were planted in the greenhouse at the Arlington Experiment Farm, Rosslyn, Va. Seed of crosses made in 1934 and in 1935 was sown the corresponding fall. The resistance of the F<sub>1</sub> plants of these crosses to the rusts and smuts of oats was studied during the corresponding winters.

In both years, the F<sub>1</sub> seed and the seed of their respective parental lines were sown in fertile soil in ordinary 5-inch greenhouse pots. Only 1 seed

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<sup>2</sup> Dietz, S. M. Inheritance of resistance in oats to *Puccinia graminis avenae*. Jour. Agr. Res. [U.S.] 37: 1-23. 1928.

<sup>3</sup> Garber, R. J. Inheritance and yield with particular reference to rust resistance and panicle type in oats. Minnesota Agr. Expt. Sta. Tech. Bull. 7. 1922.

<sup>4</sup> Selections nos. 5541, 5542, 5543, 5544, and 5556 are all from the cross X S1098, Victoria × Richland; Selection 684 is from the cross X 2868, Iowa 444 × Markton; Selection 200 is from the cross X 2737, Markton × Iogold; those from 1415 are from the Red Rust-proof strain, Cliff, and C. I. 2574 and 2592 are from the crosses Markton × Idamine and Markton × Victory, respectively.

<sup>5</sup> Parents of crosses X A1131 are Iowa 444 × Bond, of X 34 CD, C. I. 2574 × Sel. 5543, of X 3110, Anthony × O. A. C. 144, and of X 3012, Nortex × Victoria. Selection Kans. Nos. 333638, 303644, 303636, and 303635 are selections from crosses of Markton × Fulghum, and the selections Kans. Row No. 2793 is from a cross of Richland × Fulghum, X2710.





TABLE 1.—(Continued)

Cross No.	Varieties or selections crossed	Cross or parent	Total plants	Number plants smutted	Reaction of hybrid and parent plants to inoculation by smut and rust									
					Type of host response to stem-rust infection					Type of host response to crown-rust infection				
					0	1	2	3	4	0	1	2	3	4
		(Sym- bol)	(Num- ber)		(Plants resistant)									
					(Plants suscep- tible)									
					(Crosses studied in 1935-1936)									
X 35 C C	Sel. 5541-101 × Gopher	X ♀	1	0	..	..	3	..	1	..	..	..	1	..
		♂	5	0	..	..	2	..	2	..	..	5	..	3
X 35 C E	Markton × Sel. 5541-101	X ♀	3	2	..	..	..	..	3	..	..	..	2	..
		♂	2	0	..	..	2	..	..	..	..	..	..	2
X 35 E C	Markton × (Seg. from X A 1131)	X ♀	2	0	..	..	..	..	2	..	..	4	..	..
		♂	4	0	..	..	..	..	2	..	..	..	..	2
X 35 F A	(Seg. X 34 CD) × (Seg. from X 3110)	X ♀	2	0	..	..	..	..	2	..	..	..	..	1
		♂	1	1	..	..	..	..	1	..	..	..	..	1
X 35 F A		X ♀	1	0	..	..	..	..	..	..	..	1	..	..
		♂	1	0	..	..	..	..	..	..	..	..	1	..
X 35 N	Kan. Sel. 333638 × Sel. 5541-104	X ♀	1	0	..	..	1	..	..	..	1	..	..	..
		♂	4	0	..	..	..	..	4	..	..	..	..	4
X 35 A L	Kan. Sel. 303644 × Sel. 5541-104	X ♀	5	0	..	..	..	..	5	..	..	..	..	5
		♂	1	0	..	..	..	..	1	..	..	..	..	..
X 35 A O	Kan. Sel. 303636 × Sel. 5541-104	X ♀	5	0	..	..	..	..	5	..	..	..	..	6
		♂	1	0	..	..	1	..	..	..	..	1	..	..
X 35 F K	Sel. 5543-104 × Ks. Sel. 303635	X ♀	6	0	..	..	..	..	6	..	..	..	..	5
		♂	1	0	..	..	..	..	1	..	..	..	..	..
X 35 D D	(Seg. from X 3012) × Ks. Sel. Row 2793	X ♀	3	0	..	..	..	..	3	..	..	..	..	..
		♂	1	0	..	..	1	..	..	..	..	..	1	..
			5	0	..	..	..	..	5	..	..	..	..	5

<sup>a</sup> In F<sub>2</sub> all bred similarly for rust resistance.

<sup>b</sup> This plant proved to be a self.

<sup>c</sup> A possible error in reading in 1934-35.

of each hybrid was sown per pot, although, usually, the seed of a given parental line was sown at the rate of 4 to 6 seeds per pot.

The methods employed in growing the plants and inoculating them with rusts and smuts were those usually followed in the experimental greenhouse culture of oats. Before inoculating with smut, the hulls were removed from the kernels of parental lines, but, because of the value and the extreme scarcity of seed, the hybrid kernels were not hulled. The seeds were blackened with smut spores and then sown. For a few days following inoculation and planting of the seed, the greenhouse temperature was maintained at from 70° to 80° F. This procedure thus far has been highly satisfactory in obtaining smut infection of oats under greenhouse conditions. The smut inoculum depended upon the parents of each individual cross. Crosses involving only those varieties adapted to the Corn Belt were inoculated with smut collected in that area. Seed of crosses involving northern midseason white, Fulghum, and Red Rust-proof types was inoculated with smuts originally obtained from oats of these respective varietal types in areas where they are grown commercially.

Before inoculating adult plants with rust in the greenhouse, a tent of muslin was erected over the plants. They were then sprayed with an atomized stream of tap water until thoroughly wet. The floors, beds, walls, and soil beneath the beds were all thoroughly wetted to help maintain a saturated atmosphere. To further provide optimum moisture conditions, the pet cocks of the steam radiators were partly opened to allow escape and condensation of steam. All ventilators of the greenhouse were closed and a temperature of 70° to 90° was maintained for 24 hours after inoculation.

Urediospores of the rust mixed with talcum powder, an innocuous dust, were then dusted over the plants by means of a syringe bulb adapted to the purpose. Talcum powder, because of its visibility, enables the operator to note the volume and distribution of the inoculum.

*Puccinia coronata avenae*, race 1, and *P. graminis avenae*, race 2, were employed each year for inoculation. The inoculum was furnished by H. C. Murphy, of the Iowa Agricultural Experiment Station, Ames, Iowa.

## RESULTS

### Reaction of F<sub>1</sub> Plants to Smut

Data presented in table 1 show that widely different oat types were used as parents in the crosses included in the study of smut and rust resistance of F<sub>1</sub> plants in 1934-35; yet not one of the 28 F<sub>1</sub> plants showed any susceptibility to smut. This was true even in those 9 crosses of which one or the other parent proved to be smut-susceptible.

Likewise, in the results obtained from the 9 crosses grown in 1935-36, although one or the other of the parents was smut susceptible in 2 of the 9

crosses, none of the  $F_1$  plants was infected. Consequently, in these 26 crosses, one or the other parent proved susceptible in 11 crosses; yet not a single  $F_1$  plant produced any smut. This absence of smut in the  $F_1$  may have been due to the fact that the seed of the parent varieties was hulled when inoculated, while the  $F_1$  seed was not. It is our opinion, however, that the dominance of resistance over susceptibility to smut in the  $F_1$  plants of these crosses was due to genetic factors for resistance rather than to the fact that the hulls were not removed from the crossed seed. The dominance of resistance over susceptibility to smut infection in  $F_1$  would, therefore, seem evident from the reaction of these crosses.

#### Reaction of $F_1$ Plants to Stem Rust

The reaction to stem rust (*Puccinia graminis avenae*, race 2) of  $F_1$  plants of 17 crosses was studied in 1934-35. These studies indicated that where both parents were resistant to stem rust the progeny also were resistant, although notably heterozygously so in one cross. In crosses in which only one parent was resistant to stem rust, the hybrid progeny were resistant, although the  $F_1$  plants of 2 crosses gave a variable reaction, in that some plants appeared to be susceptible to stem rust.

Results of studies made in 1935-36 were similar to those obtained in 1934-35, but much less conclusive. The rust epiphytotic obtained in the greenhouse in 1935-36 was of such severity as would rarely occur naturally under field conditions. Even the most stem-rust-resistant varieties, such as Richland, showed considerable infection. Record was made of the stem-rust infection observed on the  $F_1$  plants of 9 crosses in 1935-36.

In no case were both parents of any of these crosses resistant to stem rust. In 7 crosses, one of the parents was resistant; the other parent was susceptible. Study of the  $F_1$  progeny of 7 crosses showed that 4 plants were resistant and 3 susceptible. In one cross, both parents gave a susceptible reaction, yet the  $F_1$  progeny appeared to be resistant. In one cross, plants of both the parental and the  $F_1$  progeny were susceptible to stem rust.

The data obtained as the result of the 2-year study of 26 crosses indicate that the inheritance of stem-rust resistance in most cases is dominant in  $F_1$  oat plants.

#### Reaction to Crown Rust

A study of host reaction by  $F_1$  oat plants to crown rust was conducted at the same time and on the same plants inoculated with stem rust. The results, however, were less conclusive than those obtained from stem rust. In only one cross of the 17 studied in 1934-35, both parents and  $F_1$  hybrids were resistant to crown rust. In 14 other crosses, one or the other of the parents was resistant, and in 2 other crosses, one parent was heterozygously



resistant and the other was susceptible. When one parent was either homozygously or heterozygously resistant to crown rust, plants of the  $F_1$  progeny were resistant in all except 2 crosses in which one of the  $F_1$  plants was resistant, while the others were susceptible. In this cross, both parents reacted heterozygously for both crown rust and stem rust.

The results obtained in 1935-36 differed widely from those obtained in 1934-35 in that the rust epiphytotic obtained was especially severe and only the most resistant oat types gave the *resistant type* of reaction to crown rust.

A total of 9 crosses was studied. In 2 of these crosses, both the parents and the  $F_1$  progeny were susceptible to crown rust. In 6 crosses, one of the parents was resistant and the other susceptible. The  $F_1$  progeny of these combinations resulted in 3 crosses producing resistant and 3 susceptible progeny, a result very different from what might have been expected on the basis of the previous year's study. A resistant  $F_1$  progeny resulted from the cross in which one parent was resistant and the other was heterozygously resistant to crown rust. Regardless of the exceedingly heavy infection of crown rust in 1935-36, the fact remains that resistance in the  $F_1$  frequently proved to be dominant.

A notable point in connection with studies on the reaction of parent and progeny to stem and crown rusts is that, usually, the  $F_1$  plants approximated the more resistant parent in type of reaction to rust.

#### SUMMARY AND CONCLUSIONS

From a study of the reaction of  $F_1$  oat plants and of plants of each parental strain to smut and inoculation of the adult plants with spores of stem and crown rusts, the following results were indicated.

Although parents and progeny were not treated alike, resistance to smut apparently is dominant in  $F_1$  oat plants.

Resistance to stem rust usually was dominant in  $F_1$  plants, although subject to some variation.

Resistance to crown rust usually was either dominant or intermediate in  $F_1$  plants, but the apparent dominance was less pronounced than was resistance to stem rust.

Resistance to either rust in  $F_1$  individuals of certain crosses tended to approximate the type of resistance observed in the more resistant of the parental lines.

# RECENT DEVELOPMENTS IN POTATO BREEDING FOR RESISTANCE TO VIRUS DISEASES<sup>1</sup>

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## INTRODUCTION

Resistance to virus diseases in potato varieties has been observed for some time and recently it has been shown<sup>8</sup> that resistance to one of these diseases, mild mosaic, is heritable. Further evidence of this and the results of breeding for resistance to other viroses are recorded in this paper.

Experiments have shown that potato seedlings vary in their reaction to virus diseases. Some of them fail to contract the virus either by field exposure or by grafting. Others rarely contract the virus in the field, but become infected in the graft tests, still others contract the virus readily, both by field exposure and by grafting.

Since it is important to determine whether or not seedlings will become infected naturally, field exposure as well as grafting were used in breeding for disease resistance.

## METHODS OF INOCULATION

*Field exposure.*—In the field exposure tests 10 to 20 hills of each seedling or variety were planted in rows adjacent to a row of mosaic plants, so that transmission by insect vectors was favored. Healthy Green Mountains were planted as a control with each 10 lots of seedlings. In seasons of light aphid infestation, colonies of aphids were distributed over the mosaic plants at blossom time to supplement natural infestation. As a further aid to the spread of the disease the tops of mosaic plants were cut off and placed along the rows of the seedlings 4 weeks before harvest.

<sup>1</sup> Conducted as a cooperative project between the Bureau of Plant Industry, U. S. Department of Agriculture, the Maine Agricultural Experiment Station and the Maryland Agricultural Experiment Station.

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<sup>8</sup> Schultz, E. S., C. F. Clark, R. Bonde, W. P. Raleigh and F. J. Stevenson. Resistance of potato to mosaic and other virus diseases. *Phytopath.* 24: 116-132. 1934.

The diseased seed potatoes, which provided the inoculum of a particular virus, were propagated in isolated tuber-unit seed plots and were rogued for other viroses.

The seedlings and controls were harvested by hand, and 2 tubers per hill from 10 hill lots, or 1 tuber per hill from 20 hill lots, were reserved for planting the following season when observations on the number of infected plants were made. Inasmuch as different varieties may vary in symptom expression to the same virus, representative samples of seedlings that contracted mosaic in the test plot were grafted onto healthy Green Mountain or Green Mountain seedlings for final diagnosis of the mosaic type.

*Tuber and shoot grafts.*—Tuber grafts were made by the core-graft method. Shoot grafts were made by in-arch grafting when the plants were 6 to 8 inches above the soil.

#### REACTION OF POTATO TO LATENT MOSAIC

In 1932, 65 hills each of a Green Mountain seedling, Katahdin, and seedling 41956, were grown on Aroostook Farm, Presque Isle, Maine. The hills of each of these varieties were alternated in the row with hills of Triumph that carried latent mosaic. In another series an equal number of hills of Katahdin and the 2 seedlings were grown in rows not adjoining the latent mosaic plants. Shortly after these plants had blossomed the tops of 12 hills of each of the 3 varieties not planted next to the latent mosaic plants were brushed with latent mosaic Green Mountain foliage. At harvest 2 tubers per hill from both series of tests were reserved for planting and observation in 1933. The plants grown from these tubers were tested for latent mosaic by leaf-rubbing inoculations made on Jimson weed, *Datura stramonium* L.

The results of these inoculations disclosed that Katahdin and seedling 41956 had not contracted latent mosaic in either the foliage brushing or the contact tests. In contrast to this, 70 per cent of the plants of the Green Mountain seedling became infected when grown in contact with the Triumph variety having latent mosaic, and 33 per cent became infected when grown in contact with latent mosaic Green Mountain. Inoculation by the leaf-brushing method, using latent mosaic Green Mountain foliage as the source of the inoculum, caused 58 per cent infection. Although Katahdin failed to contract latent mosaic in either the contact or leaf-brushing tests, this variety has been infected by leaf-mutilation inoculations. Inasmuch as the Green Mountain seedling, when introduced into these exposure tests, was free from latent mosaic, these results indicate that this seedling readily contracts latent mosaic if grown in contact with latent mosaic plants, or if rubbed with foliage infected with latent-mosaic virus. These results also show that Katahdin and seedling 41956 are highly resistant to latent mosaic in field-exposure tests.

For 6 consecutive seasons 100 hills each of Katahdin and of seedling 41956 were propagated in rows adjoining rows of latent-mosaic potatoes. During this period seedling 41956 failed to contract latent mosaic, while a few plants of Katahdin and every plant of the Green Mountain seedling did contract it.

Although Katahdin contracted latent mosaic in only a few plants in the field-exposure tests, this variety easily became infected in tuber and shoot grafts onto latent-mosaic Green Mountain, and, as a result, developed severe top necrosis. In tuber grafts some of the Katahdin shoots are killed at different stages of development, while other shoots manifest necrosis and more or less irregular and interveinal light green areas. Tubers from such infected Katahdin shoots develop plants manifesting a few necrotic spots and irregular light green patches on the leaves. Subsequent shoot and tuber grafts of the mottled Katahdin onto latent-mosaic Green Mountain failed to induce top necrosis. Oortwijn Botjes<sup>9</sup> reported similar results with certain European potato varieties. To determine the breeding behavior of Katahdin with respect to its reaction to latent mosaic, 100 seedlings of Katahdin naturally fertilized were in-arch grafted onto latent-mosaic Green Mountain seedlings. Fifty-seven of the Katahdin seedlings developed top necrosis. The other 43 failed to show these symptoms. It is possible that these may have escaped infection since it was not definitely known that every Green Mountain seedling onto which these were grafted harbored latent mosaic. It is interesting to note, however, that 57 per cent of the Katahdin seedlings manifested latent mosaic as top necrosis similar to the reaction of the Katahdin parent in graft tests.

In addition to the 6-year field-exposure tests several hundred plants of S 41956 have been given the more severe inoculation tests of leaf-rubbing and grafting. Up to the present time not a single plant of this seedling has contracted latent mosaic, as shown by the failure of Jimson weed and Green Mountain seedlings to become infected when return inoculations were made to them from grafted or rubbed plants. This evidence indicates that S 41956 is completely resistant to latent mosaic.

#### REACTION OF POTATO TO VEINBANDING MOSAIC

The results of the veinbanding-mosaic resistance tests in the field are not conclusive. During the last 3 seasons at Presque Isle, Maine, only 15 per cent of the Green Mountain plants showed this disease in the field-exposure tests, some of the 20-hill Green Mountain controls escaping infection completely. In the tuber-graft tests with the veinbanding mosaic no

<sup>9</sup> Oortwijn Botjes, J. G. Attenuation of the virus of top necrosis (Acro-necrosis, healthy potato virus) and acquired immunity of potato varieties to this virus. *Tijdschr. Plantenziekten* 39: 249-262. 1933. [In Dutch. English summary, p. 260-261.]

seedling has yet been found that failed to contract the disease. It was observed, however, that the seedlings varied greatly in the expression of symptoms as a result of their reaction to the veinbanding virus. Three types of reaction were observed: (1) slightly rugose, (2) veinclearing and rugose, and (3) severe necrosis with veinclearing and rugose. In 1934 5 tubers of each of 23 different seedlings were tuber-grafted onto veinbanding mosaic seedling 42898, which shows this mosaic as a slight rugosity of the leaves. All the seedlings contracted the disease, but the infection varied from less than 50 to 100 per cent. This difference probably is not due to inherent differences between the seedlings but to failure of a healthy piece of tuber to unite closely with a diseased section. The grafted seedlings developed veinclearing and necrosis, manifestations of the more severe reactions of potato to the veinbanding mosaic virus.

In 1935, 25 seedlings were given the same test for veinbanding as that described for 1934, with very similar results. All 25 contracted the disease in from 40 to 100 per cent of the tubers grafted. Again, a great variation in symptom expression was observed. Two seedlings manifested severe streaking and rugosity, while 2 others, the progeny of a different cross, developed slightly more rugose leaves than those of the healthy plants.

The wide variation in reactions of these different potato seedlings to veinbanding mosaic indicates the possibility of producing seedlings that will be tolerant or highly resistant to veinbanding mosaic.

#### REACTION OF POTATO TO MILD MOSAIC

It was indicated previously<sup>8</sup> that the reaction of different potato varieties and seedlings to mild mosaic varied greatly and could be grouped as follows: (1) Highly resistant, (2) seldom contracting mild mosaic in the field, but becoming infected in tuber grafts, (3) manifesting milder symptoms than Green Mountain, and (4) contracting mild mosaic as readily as Green Mountain and expressing symptoms not unlike those of this variety. It was indicated, too, in the field-exposure tests of 1932, that the genetic behavior of seedling varieties differs widely, as shown by the resistance of their progenies. Of the 464 seedlings of Katahdin naturally fertilized, grown in plots of 20 hills each, only 33 seedlings, or 7 per cent of the total, contracted mild mosaic. Of the 75 seedlings of S 43752 naturally fertilized, 21, or 28 per cent, contracted the disease. In contrast to this, 54 10-hill lots of Green Mountain, planted as controls, contracted mild mosaic in every lot. A similar field-exposure test of the apparently resistant seedlings of both of these progenies has since been completed.

In this test mild mosaic was contracted in 2 per cent of the 369 naturally fertilized Katahdin seedlings, in 14 per cent of the 43 naturally fertilized

S 43752 seedlings, and in every Green Mountain check. The combined results of the 2 field-exposure tests are recorded in table 1.

TABLE 1.—*Resistance to mild mosaic as shown by the results of two field-exposure tests of the progenies of Katahdin and S 43752 naturally fertilized*

Parentage of seedlings	Varieties	Lots		
		Total	Mosaic	
			No.	Per cent
Katahdin naturally fertilized .....	464 seedlings	464	42	9
S 43752, naturally fertilized .....	75 “	75	28	37
	Green Mountain controls	89	89	100

To determine whether or not the seedlings in these progenies that failed to show mild mosaic symptoms, after 2 seasons of field exposure, carried this disease, 100 of these seedlings of the progeny of Katahdin, naturally fertilized, were in-arch grafted to a Green Mountain seedling. The results showed that none of these Katahdin seedlings had contracted mild mosaic in the field-exposure tests. These same seedlings were in-arch grafted to a mild mosaic Green Mountain seedling. Thirty-eight per cent of them contracted mild mosaic in these graft tests. It is evident from the results with this progeny that, although seedling varieties are resistant under field-exposure tests, they may be susceptible in the more severe in-arch grafting tests. The in-arch grafts have not been carried far enough to determine whether or not any of the seedlings would be found resistant in such tests. The 62 per cent of the 100 Katahdin seedlings that failed to contract the disease in these tests may not be resistant; they may have escaped, only.

In a further study of the mild-mosaic resistance of Katahdin this variety was crossed with No Blight, a variety that shows some degree of resistance to late blight. The progeny of this cross, consisting of 347 seedlings, was exposed to mild mosaic on Aroostook Farm in 1934. The results of this test are given in table 2.

These results show that 86 per cent of the progeny of No Blight  $\times$  Katahdin remained healthy, whereas every one of the Green Mountain seedlings and Green Mountain controls became infected with mild mosaic. Eight of the No Blight  $\times$  Katahdin seedlings resistant to mild mosaic were also found to be highly resistant to late blight, as shown in greenhouse and field plot tests.

Katahdin has shown resistance to mild mosaic in field-exposure tests for a number of years. That it is not homozygous for this character is shown

TABLE 2.—*Resistance to mild mosaic as shown by the progeny of a cross between No Blight and Katahdin in a field exposure test*

Parentage of seedlings	Varieties	Lots		
		Total	Mosaic	
			No.	Per cent
No blight × Katahdin .....	347 seedlings	347	48	14
Green Mountain naturally fertilized	17 “	17	17	100
	Green Mountain controls	34	34	100

by the 9 per cent susceptibles in the selfed progeny. That the character is inherited as a dominant is shown both by the selfed progeny and the No Blight × Katahdin cross, which gave a ratio of apparently resistant to susceptible seedlings of 86 to 14, which ratio may, of course, be modified somewhat in further tests with this cross.

#### Reaction of Potato Seedlings and Varieties to Spindle Tuber

In 1933, 9 hills of each of 120 seedlings, selections from 24 different crosses, in addition to 99 nine-hill lots of South American varieties and 33 nine-hill lots of Green Mountains, were exposed to spindle-tuber Green Mountains planted in adjacent rows on the University of Maryland Farm, near Berwyn, Md.

At harvest time 3 hills per lot were harvested and reserved for planting in 1934.

Observations in 1934, on the material harvested from these exposed lots, showed that 71 per cent of the seedlings, 58 per cent of the South American varieties, and 81 per cent of the Green Mountain controls had contracted spindle tuber.

In 1934, nine-hill lots of 238 South American varieties were propagated in rows adjoining rows of spindle-tuber Green Mountain. Only 74 of the South American varieties developed tubers from which 2 tubers per hill were saved for planting in 1935; 2 tubers per hill were also saved from the 35 Green Mountain control lots. The results in 1935 showed that 57 per cent of the South American lots showed spindle tuber and 89 per cent of the Green Mountains had contracted the disease.

In 1935, 880 seedlings representing selections from a number of crosses and inbred lines were tuber-core grafted onto spindle-tuber Katahdin. Since spindle tuber is transmitted easily by this method only 3 tubers per seedling were grafted. One healthy tuber per seedling and the remainder of the tubers used as the source of inoculum were planted as checks. The results

showed that 61 per cent, or a total of 537 seedlings, had contracted spindle tuber in every tuber grafted; that 38.6 per cent, or 340 seedlings, developed spindle tuber in only 1 or 2 of the 3 grafted tubers, and that 1.5 per cent, or 13 seedling lots resulting from 4 different progenies, failed to contract this disease. Since it is possible that the 13 healthy seedlings merely escaped infection, additional grafts will be made to determine whether or not they are resistant. Different seedlings showed different symptoms, varying from slightly dwarfed, somewhat spindling and sparse shoots, to severely dwarfed and curled tops. Some seedlings were so tolerant that it required critical observation to detect the difference between the diseased and normal plants. To find such wide differences between sibs in their symptom expressions is important from the plant-breeding standpoint.

### Reaction of Potato Varieties and Seedlings to Leaf Roll

In 1933 and 1934, field-exposure tests were conducted to determine the reaction of various potato seedlings, commercial varieties and South American varieties to leaf roll at the University of Maryland Farm, near Berwyn. Thus far, the results are not conclusive, for, while many of the seedlings and other varieties escaped infection, a large percentage of the susceptible Green Mountain controls also failed to contract the disease. The lack of infection in the seedling and South American varieties, therefore, could not be attributed to resistance. It will be necessary to develop a more efficient method of testing or find a location where leaf-roll spread is so general that at least every susceptible control lot contracts the disease. If better conditions for spread are not found, tuber-graft tests may have to be resorted to. The objection to using the graft method only is the possibility that some seedlings might contract the disease in such a test and might very rarely contract it in a field-exposure test, a situation analogous to that found in the resistance of Katahdin to mild mosaic. In the leaf-roll tests, as in the spindle-tuber tests, a wide variation is found in the reaction of the various seedlings to the disease. Some of the diseased plants are almost normal in appearance; others are dwarfed and discolored, and show a far greater rolling of the foliage. If varieties cannot be found that will not contract the disease, some may be found that are highly tolerant to it.

### SUMMARY

Data on the inheritance of resistance to mild mosaic and the reaction of seedlings and varieties to other viroses, such as latent and veinbanding mosaics, leaf roll and spindle tuber in field-exposure tests, as well as in tuber and shoot grafts, are recorded.

Potato varieties and seedlings vary in their reaction to viroses; some are completely resistant; some fail to contract the virus in the field, but become



infected in graft tests; others rarely contract the virus in the field but become infected in grafts; still others contract the virus readily both by field exposure and by grafts. In the experiments with latent mosaic S 41956 never contracted this disease either by field exposure leaf rubbing or in grafts in tests of several hundred plants. Katahdin was highly resistant to latent mosaic in the field, but became infected with the virus by grafting.

No definite evidence of resistance to veinbanding mosaic was found in any of the varieties or seedlings tested, but there was a great variation in the severity of symptoms produced. Some seedlings manifested severe streaking and rugosity, while others, the progeny of another cross, developed only slightly more rugose leaves than those of the healthy plants. Katahdin has been completely resistant to mild mosaic in field-exposure tests for a number of years. That this resistance is heritable was shown in (8), and further evidence confirming this is given in this paper. That Katahdin is not homozygous for this character was shown by the fact that 9 per cent of the seedlings in a selfed progeny were susceptible. The progeny of the No Blight  $\times$  Katahdin cross gave a ratio of apparently resistant to susceptible of 86:14.

A total of 120 seedlings, selections from different crosses and inbred lines, and 337 lots of South American varieties were exposed to spindle tuber during 1933 and 1934. The results showed some variation in the amount of spread found in different seedlings, but, since the control plants did not all become infected, it was difficult to determine whether the plants remained healthy because of resistance or whether it was only a case of escape. Most of the 880 seedlings, tuber-grafted with spindle tuber in 1935, became infected. It has not yet been determined whether the few that failed to become infected escaped or were resistant. It was found, however, that there is a great variation in symptoms produced on the different varieties, varying from severely dwarfed and curled tops, to such mild symptoms as to require critical observation to detect the disease.

Field exposure tests in 1933 and 1934 to determine resistance to leaf roll proved to be unsatisfactory, since only a small amount of spread took place even in susceptible control plants. Transmission by tuber grafts has shown that there is a wide variation in the reaction of the different seedlings to this disease. Some are dwarfed and discolored, and show considerable rolling, while others are almost normal in appearance.

The variations of symptoms of spindle tuber and leaf roll, as expressed in different seedlings, indicate that if varieties cannot be found that will not contract these diseases, some may be found that are highly tolerant to them.

# THE NEED OF PERMANENT REFERENCE COLLECTIONS OF INSECT VECTORS OF PLANT DISEASES

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Insect collections were originally formed, maintained, and built up purely for taxonomic purposes, and large collections are now being amassed with this object primarily in mind. In a recent paper, however, Osborn (15) has pointed out various needs for entomological collections, in addition to those of pure taxonomic research, in which attention to taxonomic problems is fundamental to permanent advances in related branches of science. He also has stressed the importance of insect collections, with their type specimens, when species described by earlier workers are under investigation, since such species often are described so inadequately that they cannot possibly be identified solely from the descriptions. This is especially true in cases where discovery of related forms has brought to light many species any one of which would meet the specifications given in the early description.

At the present time our lists of known insect species are rapidly lengthening. Taxonomic workers recognize the probability that in future years many of our recently written descriptions of insects may prove inadequate for separating them from closely related forms that will doubtless be discovered, just as descriptions made a century or less ago often are found too brief for recognition of the species today. As pointed out in later paragraphs, the misidentification of species already described or the lack of agreement among taxonomists adds to the confusion.

Most entomologists realize that problems will arise from the continuously increasing literature of descriptive entomology and also the present taxonomic confusion, particularly in certain groups of insects, and some of the workers are now taking steps to preserve, for future reference, representatives of the forms upon which they are conducting biological or other studies. Such specimens will be permanently available as a means of identifying the species upon which their published work was based.

In the study of virus diseases of plants, the importance of the relationship of insects to the spread of the causal viruses is becoming more generally recognized. Kunkel (12, p. 350), in 1928, stated that "the most important factor in the spread of virus diseases is their transmission by insects. Many have been proved to be so transmitted and it is probable that all are spread in this way. . . . The more these maladies are studied, the more evident it becomes that epiphytotics of virus diseases are related to the fortunes of

insect colonies of the species concerned in the transmission of these diseases." Johnson and Hoggan (10, p. 330) indicated their realization of the importance of the specificity of insect vectors when they stated that the specific vector concerned is frequently the main criterion for identification of a plant virus. Storey (20) further emphasizes the importance of the insect in relation to certain virus diseases and cites a number of cases in which the virus is most readily identified by means of the insect vector. In separate papers Elze (5) and Storey (20) suggest classifications of viruses on the basis of the insect vector. In recent papers Kunkel (13) and Leach (14) discuss the relation of insects to the classification of plant diseases.

A resolution taking steps toward laying the foundation for an adequate classification of plant viruses, recently adopted by The American Phytopathological Society (3), indicates that a study of their insect vectors should be included. At the present time confusion exists as to the identity of certain insects that have been reported as being vectors of virus diseases.

Allard (1, p. 27) reported that *Macrosiphum tabaci* (Pergande) transmitted the mosaic disease of tobacco. No aphid by this name is known, and Patch (16, p. 29) states that *Macrosiphum tabaci* (Pergande) is a synonym of *M. solanifolii* (Ashmead). The "types" of *tabaci* are in the National Museum collection, and P. W. Mason states that they are undoubtedly the same species as *solanifolii*. In a later paper Allard (2, p. 627) stated that *Myzus persicae* (Sulz.) also transmitted tobacco mosaic. Hoggan (7, 8) found that *M. persicae* failed to transmit the virus of true tobacco mosaic from any host. *Macrosiphum solanifolii* transmitted this virus from tomato but not from tobacco. The same author found that both species of aphids readily transmitted the virus causing cucumber mosaic to all susceptible hosts and, on the basis of the insects concerned, concluded that this virus might have been the one, instead of true tobacco mosaic virus, that Allard reported as disseminated by the aphids. Johnson (9, p. 747), however, intimated that, since true tobacco-mosaic virus is highly infectious, mechanical transmission could have accounted for the spread of this disease. On the basis of work by Hoggan (7, 8) and Johnson (9), it is possible that Allard was concerned with either one or both of the viruses, depending upon the precautions he took in conducting the insect-transmission tests.

In America several viruses have been found to be transmitted by *Macrosiphum solanifolii*, while in England *M. gei* (Koch) is reported as transmitting what appear to be the same and also other viruses. Theobald (21, p. 108) reported that *M. solanifolii* was a synonym of *M. gei*. On this basis K. M. Smith (18, pp. 109, 113, 117), in his summary of the virus diseases, grouped the American work on viruses reported to be transmitted by *solanifolii* with the European work on the same problem under the one species name, *M. gei*. More recently, Hille Ris Lambers (6), after studying Theo-

bald's aphid collection, concluded that *M. gei* and *M. solanifolii* were distinct species. Because of the differences in the ability of closely related species of aphids to transmit plant virus diseases (8, 20), it seems unwise to lump this work until the matter of specific identity has been positively settled.

The vector of tomato spotted wilt in Australia originally identified as *Frankliniella insularis* (Franklin) is now considered to be an undescribed new species (19).

The vector of the virus causing aster yellows in America was formerly called *Cicadula sexnotata* (Fallén), an insect that is generally distributed in Europe, but the aster yellows is not known in Europe (11, pp. 655, 699). Dorst (4, p. 40) showed that the vector is a different species, *C. divisa* (Uhler).<sup>1</sup> According to our present knowledge *C. sexnotata* occurs only in Europe and *C. divisa* only in America, which appears to explain why aster yellows is a problem on this continent and not in Europe.

In certain papers the insect vector has been identified only to the genus, or even only to the family or order (17, p. 20), which of course lessens the value of the work from an entomological as well as a pathological standpoint.

No adverse criticism is intended of any work herein cited or of the many other references of a like nature that could have been included, for circumstances often are such that a worker cannot obtain identification of insect material before his paper goes to press, and in some cases the taxonomy of the group to which the insect belongs is in a chaotic condition.

Little uncertainty exists as to the identity of some species of insect vectors, but there is much confusion in other instances, and the question regarding the particular species concerned appears to be much involved and a subject of controversy.

To be certain of the specific identity of an insect vector is just as important in the problems of identification and means of dispersion of a given virus disease as in those problems presented by any other injurious insect. Whenever, in pathological experiments, the ability of an individual insect to transmit a particular virus<sup>2</sup> has been demonstrated, the insect involved seems to possess a value to the virus problem approaching that of a type as designated by a taxonomist describing a new species, and representatives of such specimens should be preserved for future reference. This is especially important when there is any doubt as to the identity of the species represented, or when it belongs to a group in which related species might be confused.

<sup>1</sup> In a more recent paper now in manuscript form, Dorst states that this species should be known as *Macrosteles divinus* (Uhler).

<sup>2</sup> The author agrees with the following comment by Dr. F. W. Poos, who reviewed this manuscript: "Although the virus diseases are admittedly most concerned, much of the material presented applies as well to bacterial and fungous diseases."

In the publication of the results of transmission tests an author could indicate the location of representatives of the experimental insects together with the accession number or other data by which the material may be located. The insects selected for preservation could be taken from any of the controlled experiments where positive results were obtained, particularly if from pure-line-bred stock. Whenever insects are submitted to the taxonomist for identification, ample material should be included, so that he will have no excuse for not returning duplicates if the experimenter is interested in having them for reference or exchange.

The larger entomological museums are becoming better equipped to preserve insects such as those used in biological, control, or virus studies, and facilities are improving whereby loans can be arranged, or comparisons made with insects of other countries. Since much of the work with insect transmission of virus diseases is comparatively recent, and since many of the original workers in this field are still actively engaged on their problems, it appears that most representatives of the known insect vectors of the many plant diseases could be made available for safekeeping and future reference in designated museums.

It seems particularly desirable that one or more leading institutions conducting research on virus diseases should bring together representatives of all available bona-fide, present-known insect vectors of plant viruses and serve as repositories for such material from future investigators. It is further suggested that individual workers keep available in their laboratories representative material with which they have worked until proper repositories become available.

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## SOME EFFECTS OF THE ENVIRONMENT ON THE SPONGY DRY ROT OF APPLES<sup>1</sup>

STEPHEN DIACHUN<sup>2</sup>

(Accepted for publication August 10, 1936)

A close similarity has been reported between the common black rot of apple fruit and the spongy dry rot. Stevens and Hall<sup>3</sup> stated in their original description of the spongy dry rot that "unless the spot be quite old, the disease might readily be considered to be the ordinary black rot, caused by *Sphaeropsis*, and doubtless often passes for it". They point out that in the older spots the pimples are beset with stiff black hairs that "constitute the distinctive character of this disease and serve to separate it with ease and certainty from the *Sphaeropsis* rot".

The experiments here reported show that these distinguishing setae are not always present and that environmental conditions may be important in influencing the nature of the symptoms. Under some conditions the two diseases are very similar, while under other conditions they can be recognized and separated readily. Stevens and Hall named the causal agent *Volutella fructi*, and the rot is still often called the *Volutella* rot. Saccardo, however, transferred the fungus to the genus *Colletotrichum*, due to the fact that it has black setae and acervuli that are at first subcuticular.<sup>4</sup> According to Duke the genus *Volutella* is distinct from *Colletotrichum* because *Volutella* "has a superficial origin and small oval spores and hyaline setae."<sup>5</sup> Therefore, sensu Duke and Saccardo, the fungus is not *Volutella fructi* but *Colletotrichum fructus* (Stevens and Hall) Saccardo, because it does form black setae and does not have oval spores, and consequently the name *Volutella* rot is a misnomer.

The spongy dry rot occurs in widely scattered regions. It has been reported in Massachusetts, Connecticut, Pennsylvania, New York, North Carolina, Ohio, Illinois, West Virginia, and Canada. Considerable storage

<sup>1</sup> Portion of a thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Botany in the Graduate School of the University of Illinois, 1935.

<sup>2</sup> The writer wishes to express grateful acknowledgment for the suggestions, criticism, and advice given by Drs. Const. J. Alexopoulos, F. L. Howard, C. F. Hottes, and N. E. Stevens.

<sup>3</sup> Stevens, F. L., and J. G. Hall. Some apple diseases. North Carolina Agr. Expt. Sta. Bull. 196, 1907.

<sup>4</sup> Saccardo, P. A. *Sylloge fungorum*. 22: 1201. 1913.

<sup>5</sup> Duke, M. M. The genera *Vermicularia* Fr. and *Colletotrichum* Cda. Brit. Mycol. Soc. Trans. 13: 156-184. 1928.

rot was caused in Massachusetts<sup>6</sup> where the disease has been prevalent on unsprayed and wild fruit. According to Peairs and Sherwood it caused severe damage on northwestern apples in West Virginia in 1923.<sup>7</sup>

*Colletotrichum (Volutella) fructus* (Stevens and Hall) Saccardo, has been known to occur on apple fruit; it has been observed on twigs occasionally, but has not been reported on leaves. Experiments carried out under greenhouse conditions now show that the fungus does parasitize apple leaves under some conditions.

#### METHODS AND MATERIALS

In the present experiments Grimes Golden, Willow Twig, and Jonathon varieties of apple fruit were used in the studies on symptoms. Observations were based on apples inoculated artificially. Inoculation was obtained by puncturing the skin with a sterile needle, and introducing a water suspension of spores into the wound thus made; the inoculated fruit was incubated at room temperature for 48 hours.

In order to study the susceptibility of twigs and leaves under greenhouse conditions, young seedlings were grown from seeds of Grimes Golden apples. The plants were inoculated by spraying the leaves with a water suspension of spores. Some leaves were first wounded with a sterile needle, while others were not injured. The plants were then incubated in a moist chamber 48 hours. In addition to leaves of seedlings 4 weeks old, and 2 months old, mature leaves of seedlings 2 years old were inoculated in this manner.

The susceptibility of young twigs of seedlings 6 weeks old was determined by wounding the stem with a sterile needle, applying a spore suspension, and incubating in a moist chamber at room temperature.

#### OBSERVATIONS AND CONCLUSIONS

Under average laboratory conditions of moisture and temperature the inoculated apples developed symptoms as described by Stevens and Hall<sup>8</sup>: a firm, brown, more or less circular spot was formed 5 to 7 days after inoculation; typical setose acervuli were added to the outside of those originally formed.

Usually these setose acervuli suffice to distinguish the spongy dry rot from other diseases; but it was observed that often such acervuli were not produced. Instead, small, rounded, slightly raised, blister-like bodies were formed, which produced neither spores nor setae. At such times the brown rotted area turned dark brown or black, and the spongy dry rot then strongly

<sup>6</sup> U. S. Dept. Agr. Bur. Plant Indus. Plant Disease Repr. Sup. 14: 60. 1921; 70: 204. 1929. [Mimeographed.]

<sup>7</sup> Peairs, L. M., and E. C. Sherwood. Orchard Spraying. West Virginia Agr. Expt. Sta. Circ. 36. 1924.



resembled the common black rot of apples (*Sphaeropsis malorum* Peck). The two diseases could be confused under these conditions, and, of course, could not be separated on the basis of setae, for setae were lacking. At low humidities, approximately 40 per cent relative humidity, setose acervuli were never observed; at high humidities, at or near saturation, setae usually were produced. However, because a few cases were observed where setae were not formed even under moist conditions, it seems that the amount of atmospheric moisture present is not the only conditioning factor, although it apparently is an important one. Even though all the reasons are not known, it was evident that there is a marked variation in the type of symptoms produced by the spongy dry rot of apples.

When leaves were sprayed with a water suspension of spores of the fungus, brown, round or irregular spots appeared 3 or 4 days after inoculation. If the plants were kept in a moist chamber the diseased area increased in size rapidly, and abundant acervuli were formed 5 or 6 days after inoculation. In several cases acervuli were present within 4 days. In a moist atmosphere the leaf became soft and flaccid and at times exuded a brown liquid in the late stages of decay. After the leaf was infected, the fungus often penetrated the petiole, and the entire leaf drooped; acervuli appeared on the petiole, as well as on both upper and lower surfaces of the blade.

The stems of the 6-week old seedlings which were inoculated, became brown and produced numerous acervuli; the fungus sometimes spread into the petiole and the basal portion of nearby leaves, and developed characteristic acervuli.

Apparently the fungus penetrates leaves directly through the epidermal layer, for healthy leaves were infected by simply applying a water suspension of spores to the leaf surface, either upper or lower. Microscopic examination showed that spores were germinating on the leaf surface. After 6 hours the germ tubes were 4 to 5 times the spore length; appressoria were observed on the leaf surface, but actual penetration was not seen. Evidently penetration may occur directly through the epidermal layer, for stomata are not present on the upper surface of the leaf.

Experiments show that a relatively high humidity is necessary for the development of leaf spots. In moist air the spots enlarged rapidly, sometimes involving the whole leaf in several days; but when the inoculated plants were removed from the moist chamber to dry laboratory air, at approximately 40 per cent relative humidity, the small spots increased in size but very slowly, and acervuli were not formed. If the plants were kept in the moist chamber until the leaves were spotted and then were removed into the dry laboratory air, infected leaves showed a tendency to curl, pucker, and dry. The diseased areas increased in size slightly, but fruit bodies did not develop.

For the appearance of the disease on apple leaves, and for the formation of the setose acervuli on the fruit, it seems that under laboratory conditions, a high degree of atmospheric moisture is necessary.

#### SUMMARY

The spongy dry rot of apples, *Colletotrichum fructus*, resembles the ordinary black rot under some conditions, when the formation of setose acervuli is prevented.

Leaves and twigs of seedlings grown from seeds of Grimes Golden apples, artificially inoculated, became infected by *Colletotrichum fructus*; setose acervuli were produced on such leaves and twigs.

Penetration by the fungus into the leaves seems to occur directly through the epidermis.

High atmospheric humidity is necessary for the formation of acervuli on fruit, and for the development of the disease on leaves.

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## PHYTOPATHOLOGICAL NOTE

*Black Ear Rot of Corn.*—Recently,<sup>1</sup> the junior author, while examining pure lines of corn, found that some of them were generally and severely affected with a black rot (Fig. 1). The affected ears were brought into the laboratory, where isolations were made from the blackened kernels after they were treated for 10 minutes with concentrated sulphuric acid. These isolations uniformly produced growth of a *Helminthosporium*, often in pure culture. So far as could be determined, the fungus is *H. turcicum* Pass., commonly found causing leaf blight of corn.

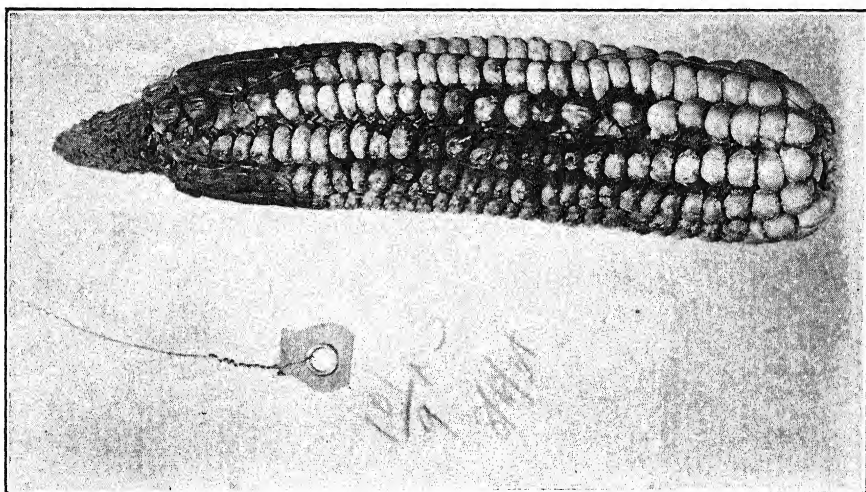


FIG. 1. Black ear rot of corn caused by *Helminthosporium turcicum* Pass.

The available literature shows no previous record of *Helminthosporium* causing ear rot of corn; hence it was thought that this note might be of interest to other plant pathologists. Since the disease has been observed in only 5 pure lines belonging to only 3 families of highly inbred corn (now in  $F_7$ ), out of nearly 500 pure lines, belonging to 74 families, all of one variety (Neal's Paymaster), the subject may be of interest also to plant breeders.—C. D. SHERBAKOFF AND L. S. MAYER, University of Tennessee, Agricultural Experiment Station.

<sup>1</sup> August 6, 1931. The delay in publishing this note is due to the authors' hope of a further study of the subject—a hope that at present cannot be realized.

## BOOK REVIEW

Wolf, Frederick A. *Tobacco Diseases and Decays*. 454 pp. 111 figures. Duke University Press, Durham, N. C. 1935. \$5.00.

It is fitting that the first comprehensive publication on the diseases of tobacco should be written by a professor of botany of Duke University and published by the press of that University. The University is situated in the flue-cured tobacco district of North Carolina. The soil in this district is of low fertility, necessitating a heavy application of a well-balanced fertilizer each year in order to grow a crop of the proper quality. This has afforded the author an excellent opportunity to become acquainted with the mineral requirements of tobacco, as well as to observe the effect of excess elements on the crop. This is an excellent region for study of the numerous bacterial and fungus diseases to which tobacco is subject, especially the root diseases. The biological activities are apparently so low in this sandy soil that there is little or no inhibition of the soil-borne pathogens. The author has not neglected the opportunity to obtain first-hand information on the numerous diseases of tobacco occurring in that vicinity. His familiarity with foreign languages and his excellent mycological background have aided him in bringing together the foreign literature on tobacco diseases. The material is well evaluated and written in a clear style. The book may be considered an authoritative source of information on the present status of tobacco diseases.

The volume opens with a short discussion of the nature and kinds of plant diseases, based upon causes, followed by a classification and a short and very interesting history of tobacco. Then follows a discussion of seed-bed practices in relation to control of diseases. Included is a discussion of the conspicuous non-pathogenic fungi that may be found in the plant bed.

A chapter on "Nutritional Diseases" follows. The inadequacy of the present methods of studying plant requirements is pointed out. The rôle of the essential elements in plant nutrition is discussed, where known, and also the effect of insufficient as well as excessive amounts of essential elements, and of several nonessential elements, on the tobacco plant.

Chapters follow on "Diseases Due to Unfavorable Water Relations" and "Disorders and Diseased Conditions of Tobacco That Are Little Known and Noninfectious." The latter chapter brings together many minor troubles, but fails to mention chlorosis of seedlings caused by cold, a condition quite common in plant beds north of the region in which the author resides, and one which, following partial recovery, is sometimes mistaken for mosaic. The discussion of frenching contains much new material originating in the laboratory of the author. Included is a histological study of frenched

leaves and considerable data on frenched and healthy plants. The author quite properly draws no conclusions as to the cause of frenching.

The chapter on virus diseases includes a comprehensive discussion of virus diseases of tobacco the world over. The discussion of tobacco mosaic is the most complete the writer has seen, and is well-balanced. It includes an excellent discussion of the epiphytology of mosaic, based largely on original research and the excellent work of Lehman. While recognition is given to the part that smoking and chewing tobacco play in initial plant-bed and field infection, undue stress appears to have been laid on manufactured tobacco as a source of mosaic, while no mention is made of cured tobacco taken directly from the barn. In the Burley and dark-tobacco areas of Kentucky, Tennessee, Virginia, and neighboring States, barn-cured tobacco is commonly used for pipe smoking and chewing by tobacco growers, and constitutes a much more extensive source of mosaic than manufactured tobacco. There is good evidence that manufactured tobacco may be substituted for barn-cured tobacco with marked reduction in the incidence of the disease. The discussion on mosaic is sufficiently complete and so clearly stated that an intelligent person, after studying it, should have little difficulty in determining what has occurred in any severe outbreak of mosaic and in pointing out means of future prevention.

In the discussion of the tobacco-mosaic virus, the author seems to have fallen into the error of accepting the host range established by Elmer for viruses of Solanaceae as that of the tobacco-mosaic virus. Elmer undoubtedly worked, in part at least, with the group of cucumber-mosaic viruses. The author might better have confined host range of tobacco-mosaic virus to that given by Grant, whose paper is not listed in the references on tobacco mosaic, but is listed in the more complete bibliography at the end of the book. A mistake was made in separating the discussion of "yellow mosaic" from that of tobacco mosaic, as yellow mosaic appears to be caused by one or another of several strains of the tobacco-mosaic virus carried in solanaceous weeds. The author properly separated cucumber-mosaic viruses from the tobacco mosaic group rather than include some of them, as was done by<sup>1</sup> K. M. Smith in his "Recent Advances in Plant Viruses," (p. 358). A comprehensive review of the literature on other virus diseases of tobacco is included. The suggestion that kromnek and corcova are identical diseases seems logical. The delphinium virus might better have been discussed with the cucumber-mosaic viruses, a group of which commonly affect tobacco in the field. Coarse etch should not have been included with the etch viruses, as it has little in common with them, except the name.

<sup>1</sup> Smith, K. M. Recent advances in plant viruses. 423 pp. J. & A. Churchill, London. 1933.

The "Bacterial Diseases" are grouped in one chapter. The discussions are not merely a compilation of published material, for the author is very familiar with several of the diseases discussed as well as the pathogens concerned. The author does not agree with Stapp that *Bact. tabacum*, *Bact. angulatum* and *Bact. melleum* are identical. Certainly, anyone who has worked with the leaf spots must recognize at least two distinct diseases, even though the pathogens are shown to be closely related. While the author is usually very cautious in his statements where controversial matter is concerned, he states that "observations, generally, are in accord in showing that wildfire invariably begins in the seed bed and is introduced into the field at time of transplanting." The bacterial leaf-spot diseases found in other countries are discussed, and an evaluation made of the evidence for the existence of distinct diseases.

The "Fungus Diseases" are considered in a single chapter. The discussions are very comprehensive. In view of the fact that the author has personally worked with nearly all the important fungus diseases considered, the discussions may be accepted as representing the latest and most authoritative information available. It might have been better if he stated that the organism responsible for the black root-rot disease of tobacco is commonly regarded as *Thielaviopsis basicola* rather than *Thielavia basicola*, as the former name is now commonly accepted. The discussion of downy mildew would have been improved had the full-page illustration used in The Bulletin (North Carolina Department of Agriculture, 1934) been used rather than or in addition to the figures after Spegazzini. The statement that White Burley tobacco, which is resistant to black root rot, is reported to be resistant to fusarium wilt is misleading.

A short chapter is devoted to nematodes, and a chapter to parasitic seed plants. *Orobanche ramosa* is the common broom rape attacking tobacco in Kentucky, and not *Orobanche ludoviciana*, as the author states.

The final chapter is devoted to "Decays of Tobacco During Curing, During Fermentation and Storage, and After Manufacture." There is a 50-page "Bibliography of Tobacco Diseases and Decays." This includes the list of references that follow the discussion of each individual topic.

The book is well written, well illustrated, and is printed on good paper. It is exceptionally free from errors, although a few appear. For example, Nolla, Guggenheim, and Roque, rather than Nolla and Roque, are credited in three places for the report on a mosaic-resistant tobacco. The book should be in the libraries of county agents in tobacco-growing areas, of field agents of cooperative and commercial tobacco organizations, of more intelligent tobacco growers, of high-school teachers in agriculture in tobacco-growing areas, and will be indispensable to research workers on tobacco diseases and to teachers of general Plant Pathology.—W. D. VALLEAU, Kentucky Agricultural Experiment Station, Lexington, Ky.

# PHYTOPATHOLOGY

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## ZÜCHTUNG PHYTOPHTORAWIDERSTANDSFÄHIGER KARTOFFELSORTEN

F. F. SIDOROV

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Die Züchtung widerstandsfähiger Sorten wurde seit Mitte des vorigen Jahrhunderts (Klotsch, Stuart) durch Anbau von Sämlingen, erhalten durch natürliche Selbstbestäubung der Kultursorten, und durch Sorten und Artenkreuzungen betrieben.

Die in erster Zeit viel angewendete Auslese von Sämlingen, erhalten durch Selbstbestäubung, wurde seit Ende des vorigen Jahrhunderts immer mehr durch die Hybridisation verdrängt. Aber trotz der vielen Arbeiten, die auf diesem Gebiete schon durchgeführt worden sind und die auch einige gute Ergebnisse gezeitigt haben, kann die Frage der Züchtung Phytophthora-fester Kartoffelsorten noch lange nicht für gelöst angesehen werden. Der Grund hierfür liegt in der "unvollkommenen Kenntnis des vorhandenen Ausgangsmaterials und der Zufälligkeit und Begrenztheit seiner Auswahl" (Bukasov). Der Artikel Hollrung's (4) "100 Jahre Kartoffelkrankheit" liefert einen schlagenden Beweis dafür; es lässt sich daraus ersehen, dass als Ausgangsmaterial für die Kreuzungsarbeiten hauptsächlich Arten Verwendung fanden, die nach unserer jetzigen Erfahrung phytophthoraanfällig waren, während die Arten, die für Immunitätszüchtungen ein grosses Interesse bieten, nicht genug herangezogen wurden.

Eine grosse Änderung wurde auf diesem Gebiet in UdSSR durch die Arbeiten des Instituts für Pflanzenbau erzielt, welches unter der Leitung von Dr. Vavilov dank seinen Expeditionen grosse Kollektionen von landwirtschaftlichen Kulturpflanzen anlegen konnte; besonders erfolgreich erwies sich diese Arbeit für die Kartoffel. Als Endergebnis konnte eine Reihe neuer Kartoffelarten festgestellt werden, sowie die bunte Mannigfaltigkeit der Kartoffelarten und Formen systematisiert und ihre Verbreitung angegeben werden (Fig. 1). Diese grosse Arbeit, die unter der Leitung von Dr. Bukasov geleistet worden ist, ermöglicht die Anwendung neuer Methoden bei der Kartoffelzüchtung in UdSSR.

Im Institut für Pflanzenbau wurde die Arbeit gleichzeitig in zwei Richtungen geführt; (1) die Erforschung der Widerstandsfähigkeit gegen *Phytophthora infestans* des im Institut aus aller Welt angesammelten Kar-





toffelmaterials zur Feststellung des Wertes dieser Kollektion in unseren Verhältnissen und (2) die Abschätzung dieses Materials zum Ausnutzen als Ausgangsmaterial bei den Kreuzungen mit unseren Kultursorten. Diese Arbeiten wurden in der Nähe von Leningrad an der Versuchsstelle des Instituts "Krasny Pachar" durchgeführt.

CHARAKTERISTIK DER KARTOFFELKOLLEKTION DES INSTITUTS UND IHR VERHALTEN GEGENÜBER PHYTOPHTHORA INFESTANS DE BARY

*Methodik der Arbeit und angewendetes Material.*—Die Prüfung des Pflanzmaterials wurde (1) unter Anwendung künstlicher Infektion im Gewächshaus, und (2) durch Abschätzung der Anfälligkeit im Felde durchgeführt. Den Freilandsbeobachtungen wurde die ganze Kartoffelkollektion der Kartoffel-Abteilung—des Instituts für Pflanzenbau unterworfen, die zur Vermehrung angepflanzt worden war, wogegen künstlich nur die Arten und Formen dieser Kollektion infiziert wurden, die grösseres Interesse für die Immunitätszüchtungen darboten.

Im Jahre 1934 wurden Stecklinge dieser Kartoffelarten nach ihrer Bewurzelung im Gewächshause mit einer Suspension von Konidien der *Phytophthora infestans* infiziert. Die Konzentration der Suspension betrug 5 Konidien im Gesichtskreis des Mikroskops bei einer Vergrößerung von 125 mal. Während des Versuches wurde im Gewächshaus für eine Temperatur und Feuchtigkeit gesorgt, die für die Entwicklung des Pilzes günstig war ( $t^{\circ}$  18°–22°,  $-h\%$  95–100).

Die Bestimmung der Anfälligkeit wurde im Moment des stärksten Befalles auf Grundlage des Vierballsystems durchgeführt. Bei der künstlichen Infektion wurde ausserdem noch der Zeitraum vom Auftragen des Infektionsstoffes bis zum Erscheinen des ersten Myzels und vom Auftragen des Infektionsstoffes bis zum Höchstpunkt der Parasitenentwicklung (der meistens mit dem Absterben der Pflanze zusammenfiel) angemerkt.

In der Regel stimmten die Ergebnisse der Schätzungen im Freiland und im Gewächshaus überein, daher wurden die Angaben der künstlichen Infektion der grösseren Genauigkeit wegen, als Grundlage für die Analyse des Materials benutzt.

Die erhaltenen Angaben gestatteten eine Feststellung des relativen Wertes der verschiedenen Kartoffelarten, Formen und Sorten in Bezug auf die Widerstandsfähigkeit gegen *Phytophthora infestans*.

Das Material wurde zur Erleichterung der Analyse in 3 Gruppen aufgeteilt:

- (1) *Solanum tuberosum*.
- (2) Die neuen Kulturarten der Kartoffel des Andengebietes von Südamerika.
- (3) Die wilden Arten.

TABELLE 1.—Das Verhalten von *Solanum tuberosum* (Handelssorten) gegen *Phytophthora infestans* bei künstlicher Infizierung

Benennung der Sorten	Erstes Erschei- nen des Myzels nach	Höchstpunkt der Entwicklung von <i>Ph. infestans</i> am	Bewertung des Befalls nach dem Vierballsystem.
	<i>Tagen</i>	<i>Tage</i>	
<i>Early Rosea</i> .....	2	5	4
<i>Early Beauty of Hebron</i> .....	2	5	4
<i>Mikado</i> .....	2	5	4
<i>Vermont</i> .....	2	6	4
<i>Dakota Red Rose</i> .....	5	9	4
<i>Charkowsky</i> .....	5	6	4
<i>Early Six Weeks</i> .....	2	5	4
<i>Kaiserkrone</i> (krebbsfest) .....	5	6-9	4
(Kuckuck, Eureka, Earliest of All)			
<i>Snezhinka No. 2</i> .....	5	6	4
<i>Magdeburger blaue</i> .....	2	9	4
<i>Goldapfel</i> .....	5	9	4
<i>Green Mountain</i> .....	5	9	4
<i>Empire State</i> .....	5	9	4
<i>Imperator</i> (Richted's) .....	5	9	4
<i>Rodensteiner</i> .....	5	7	4
<i>Cygnea</i> .....	5	9	4
<i>Champion</i> .....	5	9	4
<i>Dubowka</i> (Mutant for Sorte Im- perator) .....	2	9	4
<i>Pieter</i> .....	5	9	4
<i>Lucius</i> .....	5	9	4
<i>Odenwälder blaue</i> .....	2	9	3
<i>Märcker</i> /Prolifique tardif/ .....	3	6	4
<i>Andücker</i> /Erstlinger, Victor/ .....	2	5	4
<i>Burbank Russet</i> /California Rus- set/ .....	5	6	4
<i>Uncle Sam</i> .....	3	5	4
<i>Spaulding</i> .....	5	9	4
<i>Institut de Beauvais</i> .....	2	6	4
<i>Tschugunka</i> /Mutant der Sorte Institut de Beauvais/ .....	5	9	4
<i>Up-to-date</i> /Royalty, Fin de Siècle, Longkeeper/ .....	2-5	5-9	4
<i>Agassiz Special</i> .....	5	6	4
<i>British Queen</i> .....	5	9	4
<i>Cobbler</i> .....	2	9	4
<i>Red Bliss Triumph</i> .....	2	9	4
<i>Fürstenkrone</i> .....	2	7	4
<i>Regent</i> .....	5	7	4
<i>Jubel</i> .....	5	9	4
<i>Gratiola</i> .....	5	9	4
<i>Gloriosa</i> .....	5	9	4
<i>Pepo</i> .....	5	6	4
<i>Parnassia</i> .....	5	6	4
<i>Gisevius</i> .....	5	6	4
<i>Lichtblick</i> .....	5	6	4
<i>Pirola</i> .....	3	5	4
<i>Rosafolia</i> .....	3	5	4
<i>Belladonna</i> .....	2	5	4
<i>Wohltmann</i> .....	3	6	4

TABELLE 1.—(Continued)

Benennung der Sorten	Erstes Erschei- nen des Myzels nach	Höchstpunkt der Entwicklung von <i>Ph. infestans</i> am	Bewertung des Befalls nach dem Vierballsystem.
	<i>Tagen</i>	<i>Tage</i>	
Roode Star .....	3	6	4
Nestor .....	3	5	4
Rubia .....	2	5	4
Gracya .....	5	7	3
Gustav-Adolph .....	2	5	4
Hortensia .....	7	9	3
Fürst Bismark .....	5	9	4
Reichskanzler .....	5	9	4
Silesia .....	5	9	4
Phönix .....	5	7	4
Redball .....	5	9	4
Model .....	5	9	3
Paul Krüger .....	5	9	4
Salatny /Mutant der Sorte Paul Krüger/ .....	5	9	2
Golden Wonder .....	5	6	4
Hilners .....	3	6	4
Znicz .....	5	6	4
Asa .....	5	9	4
Zeitgeist .....	5	9	3
Eigenheimer .....	2	9	4
Eigenheimer /Blaue Knollen/ .....	6	9	4
Bund der Landwirte .....	3	7	4
Die Welt .....	3	9	4
Seidlitz .....	5	9	4
Sickingen .....	3	6	4
Helena .....	7	11	4
Douwe Jan .....	5	9	4
<sup>a</sup> Lützow .....	.....	.....	0
<sup>b</sup> Schenkendorf .....	.....	.....	0

<sup>a</sup> Von jeder Sorte wurden 5–20 Pflanzen infiziert.

<sup>b</sup> Lützow und Schenkendorf waren im Jahre 1935 befallen.

#### I. GRUPPE S. TUBEROSUM

Für *Solanum tuberosum* wurden seinerseits auch 2 Untergruppen aufgestellt: (1) die Handelssorten von Europa und Nordamerika und (2) die Chilenischen Formen.

*Handelssorten von Europa und Nordamerika.* Von den Handelssorten wurden im ganzen 92 Sorten einer Prüfung unterworfen, von denen bei künstlicher Infektion sich 2 Sorten, Lützow und Schenkendorf, am widerstandsfähigsten erwiesen und nur einen schwachen Phytophthorabefall zeigten; 9 Sorten wurden von der Krankheit ziemlich stark befallen, starben aber während des ganzen Versuches nicht ab, 2 Sorten gingen am 12-ten Tage nach der Infizierung zugrunde und 79 Sorten starben in der Zeitdauer von 5. bis zum 9. Tage nach Auftragung der Infektion.

Die widerstandsfähigsten Sorten "Lützow" u. "Schenkendorf" stellen das Resultat der Züchtung der letzten Jahre dar; an ihrem Zustandekommen

TABELLE 2.—Das Verhalten von *Solanum tuberosum* (chilenische Formen) gegen *Phytophthora infestans* bei künstlicher Infizierung

Benennung der Formen <sup>a</sup>	Erstes Erschei- nen des Myzels nach	Höchstpunkt der Entwicklung von <i>Ph. infestans</i> am	Bewertung des Befalls nach dem Vierballsystem.
	Tagen	Tage	
<i>V. chilotanum</i> .....	5	9	4
<i>f. viride</i> .....	2	9	4
“ <i>camota</i> .....	2	9	4
“ <i>indianum</i> .....	6	9	4
“ <i>chaped</i> .....	6	7	4
“ <i>cebolla</i> .....	2	7	4
“ <i>pillicuma</i> .....	2	7	4
“ <i>mahuinhue</i> .....	2	7	4
“ <i>barmacca</i> .....	2	7	4
“ <i>thalassinum</i> .....	6	6	2
“ <i>oculosum</i> .....	5	9	4
“ <i>brachykalukon</i> .....	2	5	4
“ <i>brevipapillosum</i> .....	5	7	4
“ <i>pirikuana</i> .....	5	6	4
“ <i>coraila</i> .....	5	6	4
“ <i>enode</i> .....	3	6	4
“ <i>bastoneza</i> .....	3	9	4
“ <i>latum</i> .....	3	9	4
“ <i>montanum</i> .....	2	9	4
<i>v. villaroella</i> .....	3	6	4
“ <i>recurvatum</i> .....	2	6	4
<i>f. araucanum</i> .....	2	5	4
“ <i>conicum</i> .....	3	6	4
<i>v. elegans</i> .....	2	5	4
“ <i>multibaccatum</i> .....	2	6	4
“ <i>brevipilosum</i> .....	3	6	4
<i>f. elongatum</i> .....	5	6	4
<i>v. rubrisuturatum</i> .....	6	7	4
“ <i>crassipedicellatum</i> .....	2	5	4
<i>f. contortum</i> .....	3	5	4
“ <i>huinco</i> .....	2	6	4
“ <i>pichuña</i> .....	2	6	4
“ <i>roseum</i> .....	2	9	4
“ <i>cobra</i> .....	3	6	4
<i>v. nalca</i> .....	3	6	4
<i>f. seda</i> .....	3	5	4
“ <i>costa</i> .....	2	5	4
“ <i>nigroporosum</i> .....	2	6	4
“ <i>auriculatum</i> .....	2	5	4

<sup>a</sup> Es wurden von jeder Form und Varietät von 7–24 Pflanzen untersucht.

waren nach Rathlef (7) 22 Sorten beteiligt,—während anderseits die anfälligsten Sorten, wie Frühe Rose, Imperator (Richter), ein Produkt der früheren Züchtungsarbeiten, meistens einen einfachen und kleinen Stamm-  
baum haben. Eine Erhöhung der Widerstandsfähigkeit gegen *Phytophthora infestans*, als Resultat einer akumulativen Selektion ist offenbar möglich und findet, wie es scheint, schon jetzt in den Züchtungsergebnissen der letzten Zeit ihre Bestätigung.

Eine Erhöhung der Widerstandsfähigkeit kann aber nicht nur durch Kreuzung verschiedener Sorten erhalten werden, sondern auch durch Muta-

tion: so hatten von den drei untersuchten Kartoffelmутanten, zwei eine erhöhte Widerstandsfähigkeit (Tschugunka, Salatny) im Vergleich mit den Ausgangssorten. Dieses beweist, dass bei eingehender Erforschung des für die Züchtungsarbeiten bestimmten Ausgangsmaterials einerseits, und bei Anwendung vollkommener Prüfungsmethoden in betreff der neu erzüchteten *phytophthora* widerstandsfähigen Sorten anderseits,—der Erfolg der Arbeit um ein Beträchtliches erhöht werden könnte auch bei Sorten-Kreugungen.

*Chilenische Formen.* Tabelle 2 veranschaulicht die Tatsache, dass die Chilenischen Formen im Vergleich zu den Handelssorten bedeutend stärker von *Phytophthora infestans* befallen werden. Die botanisch-systematische Erforschung dieser Formen (Bukasov u. a.) (1, 2, 5) erlaubt deren Einteilung in 3 Gruppen, von denen jede eine verschiedene Geschichte und zugleich auch einen verschiedenen Züchtungswert hat.

Die erste Gruppe der Formen, die mit den europäischen Sorten identisch ist, wurde offenbar aus Europa nach Chile nachträglich eingeführt wegen ihres besseren Ernteertrages im Vergleich mit den einheimischen Sorten. Zu dieser Gruppe gehört nach den Angaben von Dr. Bukasov und Woskresenskaja die Form "*thalassimum*," die grosses Interesse vom Standpunkt der Widerstandsfähigkeit darbietet. Die meisten Formen bieten als Ausgangsmaterial für die Züchtungsarbeiten genetisch nichts Neues im Vergleich mit den Handelssorten.

Die zweite Gruppe der Formen hat ebenso eine Reihe von Merkmalen, die den Handelssorten eigen sind. Offenbar waren diese Sorten seiner Zeit nach Europa oder nach Nord Amerika ausgeführt, wo sie einer züchterischen Bearbeitung unterworfen wurden. Der Wert dieser Formen ist für die Züchtung auch nicht gross, da ihr Genenbestand schon seit langer Zeit von den Züchtern ausgenutzt wird. Zu dieser Gruppe gehören nach den Angaben von Bukasov (2) und Woskresenskaja:<sup>1</sup> *v. chilotanum*, *f. chaped*, und *f. oculosum*.

Die dritte Gruppe ergeben die endemischen Chilenischen Formen, die sich vollkommen von den Handelssorten unterscheiden. Diese Gruppe bietet ein grosses Interesse für die Immunitätszüchtungen gegen *Phytophthora infestans* wegen der vorauszusetzenden, die Immunität bedingenden polyfaktoriellen Natur. Das Heranziehen dieser Gruppe für die Kartoffelzüchtung könnte offenbar den Genenbestand der Kultursorten bereichern.

## II GRUPPE.—NEUE KULTURARTEN DER KARTOFFEL

Diese Arten werden in 2 Untergruppen eingeteilt: zu der I Untergruppe gehört *Solanum andigenum*, zu der II Untergruppe gehören die primitiven Arten.

*S. andigenum.* Die neue Kulturart *S. andigenum* ist für den Züchter von Interesse, infolge ihres Polymorphismus und ihrer weiten Verbreitung

<sup>1</sup> Laut mündlicher Mitteilung.

als einheimische kulturkartoffel. Nach Bukasov (1, 2) ist diese Art in Mexico, Guatemala, Kolumbien, Ecuador, Peru, Bolivien und in den Anden Argentiniens verbreitet. Tabelle 3 veranschaulicht die grossen Unterschiede in der Widerstandsfähigkeit gegen den Phytophthorabefall, welcher in bestimmtem Zusammenhang mit dem Herkunftsort der Formen steht. Bei der Einteilung der Formen nach diesem Prinzip tritt die Verschiedenheit ihres Wertes für die Immunitätszüchtungen deutlich hervor. An erster

TABELLE 3. Das Verhalten der verschiedenen Formen von *Solanum andigenum* gegen *Phytophthora infestans* bei künstlicher Infizierung

Benennung der Sorten <sup>a</sup>	Erstes Erschei- nen des Myzels nach	Höchstpunkt der Entwicklung von <i>Ph. infestans</i> am	Bewertung des Befalls nach dem Vierballsystem.
MEXICO	Tagen	Tage	
var. <i>mexicanum</i> f. <i>tolucanum</i> .....	6	9	3
“ “ f. <i>chalcoense</i> .....	2	6	2
KOLUMBIEN			
“ <i>colombianum</i> f. <i>tocanum</i> .....	3	3	1-2
“ “ f. <i>lilacinoflorum</i> .....	5	6	2
“ “ f. <i>caiceda</i> .....	3	7	2
“ “ f. <i>usme</i> .....	5	11	4
“ “ f. <i>funzanum</i> .....	5	7	4
“ <i>hederiforme</i> .....	5	7	4
CENTRAL PERU			
“ <i>juninum</i> f. <i>Jana mata</i> .....	3	5	4
“ f. <i>Carhuamayo C.</i> .....	2	11	4
“ f. <i>Jana huancayo</i> .....	5	11	4
Sortentypus <i>Curao blanco</i> .....	2	9	4
“ <i>Chinchao</i> .....	3	5	4
f. <i>nigrum</i> .....	2	6	4
f. <i>mejorada</i> .....	3	5	4
f. <i>ananasiforme</i> .....	2	6	4
f. <i>Jauja</i> .....	2	6	4
Sortentypus <i>Zamba</i> .....	2	5	4
f. <i>puca almilla</i> .....	2	5	4
SÜD-PERU			
v. <i>socco-huaccoto</i> .....	3	5	4
f. <i>chimaco</i> .....	3	5	4
f. <i>puca-papa</i> .....	3	5	4
f. <i>ckecco</i> .....	3	5	4
f. <i>ppac-nacha</i> .....	3	6	4
f. <i>pumac</i> .....	3	9	4
f. <i>lutuc runtum</i> .....	6	9	4
f. <i>ppagui</i> .....	2	3	3
f. <i>hualteo lomo</i> .....	2	11	4
v. <i>ocusi</i> .....	6	11	4
f. <i>puca</i> .....	3	5	3
f. <i>Acomayo</i> .....	3	11	3
v. <i>quechuanum</i> ( <i>Taccla</i> ) .....	2	6	3
v. <i>cuzcoense</i> .....	2	6	3
f. <i>huilca</i> .....	3	9	3
f. <i>ccorau</i> .....	5	9	3
f. <i>moraleño</i> .....	3	5	3
f. <i>uncuña</i> .....	2	11	4

Benennung der Sorten	Erstes Erschei- nen des Myzels nach	Höchstpunkt der Entwicklung von <i>Ph. infestans</i> am	Bewertung des Befalls nach dem Vierballsystem.
BOLIVIEN	<i>Tagen</i>	<i>Tage</i>	
v. <i>imilla</i> f. <i>chiar-imilla</i> .....	2	7	2
“ “ f. <i>ccompis</i> .....	4	7	4
f. <i>globosum</i> .....	2	2	1
f. <i>conicicolumnatum</i> .....	2	9	2
f. <i>lanciacuminatum</i> .....	2	3	2-3
v. <i>bolivianum</i> .....	2	9	2
f. <i>incrassatum</i> .....	5	7	2
f. <i>bifidum</i> .....	2	7	3
f. <i>arcuatum</i> .....	2	11	4
v. <i>longibaccatum</i> .....	3	9	4
v. <i>brevicalyx</i> .....	4	11	2
f. <i>cryptostylum</i> .....	2	5	4
v. <i>aymaranum</i> .....	2	7	2
f. <i>Cevallosii</i> .....	2	7	4
f. <i>compressostygmatum</i> .....	5	11	4
f. aus <i>Phitcallae</i> .....	4	11	4

<sup>a</sup> Es wurden von jeder Form 1-20 Pflanzen untersucht.

Stelle stehen dem Werte nach die Formen aus Kolumbien, von denen viele nur schwach anfällig sind. Ausser der Phytophthorawiderstandsfähigkeit ist bei vielen Formen Kolumbiens auch eine nicht allzuschlechte Knollenbildung unter den Bedingungen eines langen Tages beobachtet worden.

TABELLE 4.—Verhalten der primitiven Kulturarten der Kartoffeln gegen *Phytophthora infestans* bei künstlicher Infizierung

Benennung der Arten <sup>a</sup> und Formen	Erstes Er- scheinen des Myzels nach	Höchst- punkt der Entwick- lung von <i>P. infestans</i> am	Bewertung des Befalls nach dem Vierball- system	Anmer- kungen
	<i>Tagen</i>	<i>Tage</i>		
<i>S. phureja</i> .....	2	5	4	
<i>S. goniocalyx</i> /rote Knollen/ .....	2	5	4	
<i>S. stenotomum</i> v. <i>pinu</i> .....	2	6	4	
<i>S. Rybinii</i> .....	2	5	4	
<i>S. ajanhuiri</i> f. <i>pigmentatum</i> .....	2	5	4	
<i>S. boyacense</i> .....	.....	.....	..... <sup>x/</sup>	<sup>x/</sup> Im Freila
<i>S. chaucha</i> f. <i>pigmentatum</i> .....	3	9	4	nd stark
<i>S. “</i> v. <i>surimana</i> .....	3	9	4	befallen
<i>S. tenuifilamentum</i> .....	.....	.....	..... <sup>x/</sup>	
<i>S. mamilliferum</i> .....	2	6	4	
<i>S. chocclo</i> .....	.....	.....	..... <sup>x/</sup>	
<i>S. Juzepczukii</i> f. <i>ccaisella</i> .....	2	5	4	
<i>S. curtilobum</i> f. <i>pigmentatum</i> .....	2	5	4	

<sup>a</sup> Es wurden von jeder Art von 3-70 Pflanzen für den Versuch genommen.

Dieses alles erlaubt, die Formen aus Kolumbien ihrem Wert nach, als Ausgangsmaterial für die Züchtung an eine der ersten Stellen zu setzen.

Die nächste Gruppe ergeben die Formen aus Bolivien; in betreff der Widerstandsfähigkeit gegen *Phytophthora*, und der Knollenbildung stehen sie sehr nahe zu den kolumbischen Formen, und dürfen infolgedessen die nächste Stelle nach ihnen einnehmen.

Die Formen aus Südperu bieten ein weniger wertvolles Material, da sie weniger widerstandsfähig sind und ausserdem eine schlechtere Knollenbildung aufweisen.

Das *Solanum andigenum* aus Centralperu ist durch die am meisten anfälligen Formen vertreten.

Etwas abseits steht die Gruppe der mexikanischen und guatemalischen Formen, die annähernd ebenso widerstandsfähig, wie die Formen aus Bolivien, sind, jedoch Knollen schlechter bilden.

*Primitive Kulturarten.* Die von uns untersuchten Arten dieser Gruppe zeichneten sich durch eine starke *Phytophthora*-anfälligkeit aus. Obgleich die Untersuchungen natürlich nicht die grosse Mannigfaltigkeit dieser Arten umfassen konnten, geben sie doch die Möglichkeit festzustellen, dass diese Arten einen viel geringeren Wert im Vergleich mit *S. andigenum* für die Immunitätszüchtung besitzen.

### III. GRUPPE.—WILDE KARTOFFELARTEN

Die wilden Kartoffelarten stammen aus verschiedenen Ländern. Wie aus der Tabelle 5 zu ersehen ist, sind die *phytophthoraimmunen* Formen in Mexiko konzentriert, wo gegenwärtig 9 *phytophthoraimmune* Arten vorgefunden worden sind. Diese Arten unterscheiden sich morphologisch und physiologisch scharf von einander, da sie verschiedenen systematischen Gruppen und Untergruppen angehören, was mit Recht auch das Vorhandensein von Unterschieden in der Widerstandsfähigkeit gegen *Phytophthora infestans* voraussetzen lässt. Die Verschiedenheit der Reaktion der Wirtspflanze auf das Eindringen des Pilzes trat bei der künstlichen Infizierung deutlich hervor. So traten an einigen Arten bei künstlicher Infizierung keine Befallsanzeichen auf, während an anderen Arten die Folgen des Befalls sich in Form von charakteristischen kleinen Flecken und Schimmelrasen an den Blättern der Pflanzen bemerkbar machten. Bei den anfälligen Sorten lebt nach Hollrung (4) der Parasit und die Pflanze in einer Symbiose, bei den immunen Pflanzen jedoch kommt eine solche Symbiose nicht zu Stande. Der in die Pflanze vordringende Pilz ruft das Absterben des ihn umgebenden Pflanzengewebes hervor, woraufhin auch das Absterben des Parasiten erfolgt. Einige Forscher meinen, dass das Eindringen des Parasiten in das Gewebe der Wirtspflanze eine Reihe biochemischer Prozesse auslöse, als deren Folge bei den immunen artengiftige Stoffe entständen,



TABELLE 5.—Verhalten der wilden Kartoffelarten gegen *Phytophthora infestans* bei künstlicher Infizierung

Benennung der Gruppe	Benennung der Art und Form	Anzahl der Chromosomen	Herkunft	Erstes Erscheinen des Myzels nach	Höchstpunkt der Entwicklung von <i>Phytophthora infestans</i> am	Bewertung des Befalls nach dem Vierballsystem
			Tagen	Tagen	Tagen	
<i>Tuberosa</i>	<i>S. demissum</i> f. <i>stenanteratum</i> .....	72	Mexico	.....	.....	0
"	<i>S. demissum</i> f. <i>atrocyaneum</i> .....	72	"	.....	.....	0
"	<i>S. demissum</i> —0227 .....	72	"	.....	.....	0
"	" v. <i>thlaxpehuolcoense</i> (I) .....	72	"	.....	.....	0
"	" " " (II) .....	72	"	.....	.....	0
"	" " " (VII) .....	72	"	.....	.....	0
"	<i>S. edinense</i> .....	60	"	.....	.....	0
"	<i>S. semidemissum</i> .....	60	"	.....	.....	0
"	<i>S. Antipovicii</i> .....	48	"	.....	.....	0
"	" " v. <i>neantipovicii</i> (Gandarae) <sup>a</sup> .....	48	"	.....	.....	0
"	" " v. <i>reddickii</i> .....	48	"	.....	.....	0
"	" " v. <i>martinezii</i> .....	48	"	.....	.....	0
"	<i>S. ajuscoense</i> .....	48	"	3	3	1
"	" " v. <i>candelarianum</i> .....	48	"	2	2	1
"	<i>S. Vallis mexici</i> .....	36	"	2	2	1
"	<i>S. verrucosum</i> ( <i>squamulosum</i> ) .....	24	"	.....	.....	0
<i>Bulbocastana</i>	<i>S. polyadenium</i> .....	24	"	3	3	1
<i>Pinnatisecta</i>	<i>S. bulbocastanum</i> .....	24	"	.....	.....	0
"	<i>S. coyocacanum</i> .....	36	"	.....	.....	0
"	<i>S. Commersonii</i> .....	36	"	.....	.....	0
"	<i>S. subtilius</i> .....	36	Uruguay	2	.....	0
<i>Tuberosa</i>	<i>S. leptostigma</i> .....	24	"	11	3	2
"	<i>S. Molinae</i> .....	48	Chili	3	11	2
"	<i>S. Maglia</i> .....	48	"	2	5	4
<i>Pinnatisecta</i>	<i>S. Jamesii</i> .....	36	"	5	5	4
"	<i>S. chacoense</i> .....	24	"	3	6	4
"	.....	24	Argentina	3	5	4
			Sehr stark im Felde befallen			

<sup>a</sup> Es wird eine Spaltung nach dem Faktor der Immunität gegen *Ph. infestans* beobachtet.

TABELLE 6.—Krautfäuligkeit der FI Hybriden *S. antigenum* × *S. tuberosum* gegen *Phytophthora infestans* in Jahre 1933 bei Vermehrung durch Knollen

Benennung der Elternformen: <sup>a</sup>	Befall der Elternformen <i>S. tuberosum</i> <i>S. andigenum</i>	Anzahl der Hybriden					Gesamtzahl der Hybriden
		Bewertung des Befalls nach dem Vierballsystem					
		0	1	2	3	4	
I. Hybriden mit v. <i>hederiforme</i>	2	%	%	%	%	%	
v. <i>hederiforme</i> × Alma .....	$\frac{2}{2}$	18,2	43,2	27,7	10,3	1,2	630
Early Rose × <i>hederiforme</i> .....	$\frac{4}{2}$		1,4	10,7	80,1	7,8	561
Imperator (Richter) × <i>hederiforme</i> .....	$\frac{3}{2}$		1,3	28,2	69,2	1,3	78
Salamans Samling von der Sorte President × <i>hederiforme</i> .....	$\frac{2}{2}$		3,2	29	64,6	3,2	31
Fürstenkrone × <i>hederiforme</i> .....	$\frac{3}{2}$		17,9	44,3	37,4	0,4	486
Belladonna × <i>hederiforme</i> .....	$\frac{2}{2}$		17,0	80,2	2,8		176
Hindenburg × <i>hederiforme</i> .....	$\frac{2}{2}$		33	67			9
Zhicz × <i>hederiforme</i> .....	$\frac{1}{2}$		21	79			49
II. Hybriden mit f. <i>tozanum</i>	4			15,3	70,2	14,5	124
Early Rose × <i>tozanum</i> .....	$\frac{1}{1}$						
Alma × <i>toncanum</i> .....	$\frac{2}{1}$		15,0	69,8	15,2		63
Green Mountain × <i>tozanum</i> .....	$\frac{3}{1}$		10	20	70		29
Fürstenkrone × <i>tozanum</i> .....	$\frac{3}{1}$		7,5	38,2	50	4,3	92
Belladonna × <i>tozanum</i> .....	$\frac{2}{1}$		13,8	79,9	6,3	.....	368
Rotkaragis × <i>tozanum</i> .....	$\frac{3}{1}$		24,2	74,2	1,6		322
Hindenburg × <i>tozanum</i> .....	$\frac{2}{1}$		17,7	74,9	8,0		531
Jubel × <i>tozanum</i> .....	$\frac{1}{1}$	5,6	61,5	27,8	5,1	.....	270
							3819

<sup>a</sup> Jede Nummer war meistens durch 2 Pflanzen vertreten.

die eine Vergiftung der Pflanzengewebe und des Parasiten hervorriefen. Die Arbeiten von Dufrénoy (3) haben bei den phytophthoraimmunen Kartoffelsorten in dem Gewebe, das an die befallenen Pflanzenteile grenzte, das

Vorhandensein von Phänolverbindungen festgestellt, welche nach seiner Meinung die Immunität dieser Sorten zum Krebs und zur Kartoffelfäule bedingen.

Der wirtschaftliche Wert verschiedener wilder Arten wird ausserdem noch nach der Möglichkeit ihrer Kreuzbarkeit mit den Handelssorten bestimmt. Letzteres hängt von 2 Ursachen ab: (1) von der systematischen Nähe zu den Kulturarten und (2) von der Anzahl der Chromosomen, die der einen oder der anderen Art eigen ist—wodurch gewöhnlich die Sterilität beeinflusst wird. Zieht man dieses in Betracht, so scheinen die wilden Arten der Gruppe *Tuberosa*, die zu den Untergruppen *Demissa* und *Longipedicellata* gehören, die aussichtsreichsten zu sein und zwar *Solanum demissum* und *S. Antipoviczii*. Eine Kreuzung mit den Arten *S. semidemissum* und *S. Vallis Mexici* derselben Untergruppe gelingt gewöhnlich nicht.

Die dritte Art *Solanum verrucosum*, die 24 Chromosomen aufweist und etwas stärker von *Phytophthora infestans* befallen wurde, ist im Vergleich mit den oben genannten Arten für die Züchtung von geringerem Interesse.

#### ZÜCHTUNGSWERT EINIGER KARTOFFELARTEN

*Methodik der Arbeit und benutztes Material.* In dieser Richtung wurde die Arbeit nur mit 4 Gruppen von Hybriden durchgeführt, die in Krasny Pachar im Jahr 1931 erhalten worden waren, und zwar durch Kreuzungen:

- (1) Zwischen *Solanum andigenum* und *S. tuberosum*.
- (2) Zwischen *S. demissum* und *S. tuberosum* oder *S. andigenum*.
- (3) Zwischen *S. semidemissum* und *S. tuberosum*.
- (4) Zwischen *S. Antipoviczii* und *S. tuberosum*.

Im Jahre 1932 wurden zum ersten Mal die Sämmlinge der Kreuzungen vom Jahre 1931 in einer Anzahl von etwa 30.000 Stück ausgesetzt. Die meisten dieser Sämlinge stellten Hybriden der Kreuzung zwischen *S. tuberosum* und *S. andigenum* dar. Von den Hybriden aus Kreuzungen zwischen *S. tuberosum* und den wilden Arten war nur eine Anzahl von 6.500 Pflanzen vorhanden.  $F_1$  *S. Antipoviczii*  $\times$  *S. tuberosum* war nur durch eine einzige Pflanzen vertreten.

Die Schätzung der Stärke des Phytophthorabefalles wurde ebenfalls nach dem Vierballsystem durchgeführt.

#### I. HYBRIDEN *S. ANDIGENUM* $\times$ *S. TUBEROSUM*

Als Material für die Kreuzungen dienten *f. tocanum* und *v. hederiforme*. Systematisch steht die Art *Solanum andigenum* ziemlich nahe zu *S. tuberosum*.

*Verhalten der  $F_1$  zum Befall von Phytophthora infestans.* Auf Grund der Angaben in Tabelle 6 ist es unmöglich die Faktoren zu bestimmen, von denen die Immunität gegen *Phytophthora* abhängt, jedoch einige Folge-

rungen, die die Richtung der weiteren Arbeit bestimmen könnten, dürfen dennoch gemacht werden. Es berechtigt dazu die verhältnismässig grosse Menge des Versuchsmaterials und der Cyklus von ausgeführten Kreuzungen zwischen *S. tuberosum* und den beiden Komponenten aus *S. andigenum*.

Auf Grund der durchgeführten Arbeit konnte festgestellt werden, dass die Mehrzahl der Hybriden bei allen Kombinationen in betreff der Widerstandsfähigkeit den Elternformen nahe stand, dass sich aber der Einfluss des Komponenten aus *Solanum tuberosum* stärker geltend machte, als derjenige des Komponenten aus *S. andigenum*, infolge dessen muss beim Heranziehen von *S. tuberosum* die Widerstandsfähigkeit dieser Art besondere Beachtung finden. In beiden Kombinationsgruppen konnten in einzelnen Fällen neue Formen beobachtet werden, die der Widerstandsfähigkeit nach die Elternformen übertrafen, wodurch die Möglichkeit gegeben wurde schon im  $F_1$  eine Auslese des Materials auszuführen.

*Ertragsfähigkeit der  $F_1$  Hybriden.* Tabelle 7 zeigt, dass hier ebenso wie bei der Phytophthorawiderstandsfähigkeit eine gewisse Gesetzmässigkeit in der Ertragsfähigkeit der Hybriden dieser beiden Gruppen zu beobachten ist. Der Einfluss von *Solanum andigenum* machte sich offenbar darin geltend, dass eine grosse Anzahl der Hybriden (wie Tabelle 7 zeigt) keine Knollen oder nur sehr wenig Knollen bildeten.

*Stärkegehalt der Hybriden.* Die Aufspaltung der Hybriden nach dem Prozentsatz des Stärkegehaltes richtete sich nach denselben Prinzipien, wie bei der Aufspaltung nach den oben erwähnten Eigenschaften. Da die Angaben in den Tabellen 6, 7, und 8 keinen Aufschluss über den vollen wirtschaftlichen Wert dieser Hybriden geben, wurde der Koeffizient der Korrelation zwischen Phytophthorawiderstandsfähigkeit und Stärkegehalt der Knollen pro Staude errechnet. Ein unmittelbares Verhältnis zwischen den beiden Eigenschaften wurde nicht festgestellt, was also darauf hinweist, dass eine Koppelung zwischen Ertragsfähigkeit und Anfälligkeit nicht stattfindet und folglich die Möglichkeit einer glücklichen Kombination dieser beiden Eigenschaften in einer Sorte nicht ausgeschlossen ist. Ebenso wies die Analyse des Materials auf die Möglichkeit hin in einzelnen Fällen Hybriden zu erhalten, die auch an Form und Geschmack der Knollen, sowie in betreff der flachen Augen die Elternformen übertreffen. Insgesamt kann der Schluss gezogen werden, dass aus Kreuzungen zwischen *Solanum tuberosum* und *S. andigenum*, Hybriden erzüchtet werden können, die ihren gesamten Eigenschaften nach die Handelssorten übertreffen werden.

Zu dieser Folgerung kommt auch Rathlef (7) in seinen Arbeiten.

## II. HYBRIDEN *S. DEMISSUM* × HANDELSORTEN

*S. demissum*, welches sich durch Frosthärte und Phytophthorafestigkeit auszeichnet, wurde schon in der Mitte des vorigen Jahrhunderts von den

TABELLE 7.—Ertrag der F<sub>1</sub> Hybriden *Solanum andigenum* × *S. tuberosum*

Benennung der Elternformen <sup>a</sup>	Ertrag der Elternsorten <i>S. tuberosum</i> <i>S. andigenum</i>		Anzahl- der Hy- briden die keine Knollen bildeten in %	Anzahl der Hybriden in %															Anzahl der Hy- briden
	Durchschnittsgewicht eines Nestes in Gramm																		
	Gramm			von bis 100	von 200 bis 400	von 400 bis 600	von 600 bis 800	von 800 bis 1000	von 1000 bis 1200	von 1200 bis 1400	von 1400 bis 1600	von 1600 bis 1800	von 1800 bis 2000	von 2000 und mehr.					
	%	%		%	%	%	%	%	%	%	%	%	%	%	%				
Hybriden mit v. <i>hederiforme</i> . <i>hederiforme</i> × <i>Alma</i> .....	600 300	800 300	5.5	10.6	15.8	33.6	23.7	8.2	1.8	0.6	0.2	.....	.....	.....	670	.....	.....	.....	
Early Rose × <i>hederiforme</i> .....	800 300	800 300	5.2	4.8	15.4	25.0	24.2	12.2	7.1	3.6	1.4	0.9	0.2	.....	636	.....	.....	.....	
Imperator (Richter) × <i>hederi- forme</i> .....	600 300	800 300	28.8	3.6	16.6	20.9	16.6	8.7	3.6	1.4	.....	.....	.....	.....	139	.....	.....	.....	
Fürstenkrone × <i>hederiforme</i> ..	..... 300	..... 300	13.9	2.4	9.6	27.2	23.4	14.9	5.5	2.1	0.2	0.4	0.2	.....	502	.....	.....	.....	
Salamans Samling von der Torte President × <i>hederi- forme</i> .....	..... 300	..... 300	14.7	2.9	17.5	35.0	21.2	2.9	2.9	2.9	.....	.....	.....	.....	35	.....	.....	.....	
Belladonna × <i>hederiforme</i> .....	..... 300	..... 300	25.2	2.5	10.6	26.6	20.8	10.8	3.5	.....	.....	.....	.....	.....	199	.....	.....	.....	
Hindenburg × <i>hederiforme</i> .....	900 300	900 300	35.7	7.1	28.6	28.6	.....	.....	.....	.....	.....	.....	.....	.....	14	.....	.....	.....	
Zniecz × <i>hederiforme</i> .....	600 300	600 300	43.9	8.5	14.6	17.0	10.9	5.1	.....	.....	.....	.....	.....	.....	82	.....	.....	.....	

TABELLE 7.—Ertrag der F<sub>1</sub> Hybriden *Solanum andigenum* × *S. tuberosum*

Benennung der Elternformena	Ertrag der Elternsorten <i>S. tuberosum</i> <i>S. andigenum</i>		Anzahl- der Hy- briden die keine Knollen bildeten in %	Anzahl der Hybriden in %														Anzahl der Hy- briden	
	Gramm			Durchschnittsgewicht eines Nestes in Gramm															
				bis 100	von 100 bis 200	von 200 bis 400	von 400 bis 600	von 600 bis 800	von 800 bis 1000	von 1000 bis 1200	von 1200 bis 1400	von 1400 bis 1600	von 1600 bis 1800	von 1800 bis 2000	von 2000 und mehr.				
																%	%		%
II. Hybriden mit <i>tocanum</i> . Early Rose × <i>tocanum</i> .....	800 200		6.5	1.5	10.1	25.4	20.2	19.3	3.0	6.0	4.5	0.7	0.7	2.1	138				
Alma × <i>tocanum</i> .....	600 200		28.1	1.1	1.1	28.1	22.1	12.8	4.5	2.2	.....	.....	.....	.....	89				
Green Mountain × <i>tocanum</i> ..	..... 200		23.7	23.7	26.3	21.0	5.3	.....	.....	.....	.....	.....	.....	.....	38				
Fürstenskrona × <i>tocanum</i> .....	..... 200		21.3	3.6	18.5	25.7	9.9	14.2	2.6	2.6	1.7	.....	.....	.....	113				
Belladonna × <i>tocanum</i> .....	..... 200		36.2	1.3	9.9	25.6	19.8	4.4	1.8	0.4	0.4	0.2	.....	.....	550				
Rotkaragis × <i>tocanum</i> .....	..... 200		18.0	0.9	10.9	22.7	23.6	18.2	3.4	1.6	0.7	.....	.....	.....	433				
Hindenburg × <i>tocanum</i> .....	900 200		15.7	4.7	20.0	33.3	18.2	5.6	2.0	0.5	.....	.....	.....	.....	610				
Jubel × <i>tocanum</i> .....	800 200		23.5	2.1	4.8	23.9	22.7	13.3	5.2	2.9	1.1	0.4	.....	.....	481				
Gesamtanzahl .....															4673				

<sup>a</sup> Jede Nummer war meistens durch 2 Pflanzen vertreten.<sup>†</sup> Angaben vom Jahre 1933 bei Vermehrung durch Knollen.



TABELLE 8.—Stärkegehalt der  $F_1$  Hybriden *S. andigenum* × *S. tuberosum* nach den Angaben vom Jahre 1933 bei Vermehrung durch Knollen.—(Continued)

Elternformena Benennung der	Stärkepro- zentsatz der Eltern- sorten; <i>S. tuberosum</i> <i>S. andigenum</i>	Anzahl der Hybriden in %											Gesamtzahl der Hybriden	
		Stärkegehalt												
		bis 10 %	von 10 bis 12 %	von 12 bis 14 %	von 14 bis 16 %	von 16 bis 18 %	von 18 bis 20 %	von 20 bis 22 %	von 22 bis 24 %	von 24 bis 26 %	von 26 bis 28 %	von 28 bis 30 %		über 30 %
HYBRIDEN MIT F. TOCANUM														
Early Rose × tocanum .....	— 13	2.9	10.4	39.7	35.0	8.9	2.9	.....	.....	.....	.....	.....	.....	134
Alma × tocanum .....	12 13	1.6	17.2	29.6	40.6	7.8	3.1	.....	.....	.....	.....	.....	.....	64
Green Mountain × tocanum .....	— 13	20	4	40	20	4	12	.....	.....	.....	.....	.....	.....	25
Fürstenskronen × tocanum .....	— 13	10.9	16.3	36.9	20.6	5.5	7.5	1.1	.....	.....	1.1	.....	.....	92
Belladonna × tocanum .....	— 13	2.0	13.3	28.2	35.5	15.6	4.6	0.4	.....	0.2	.....	.....	0.2	347
Rotkaragis × tocanum .....	— 13	1.9	12.8	29.5	36.8	13.8	4.6	0.3	.....	0.3	.....	.....	.....	326
Hindenburg × tocanum .....	12.9 13	15.1	25.2	35.8	18.1	3.3	1.8	0.4	0.2	.....	.....	.....	.....	504
Jubel × tocanum .....	16.4 13	3.1	11.3	34.7	34.9	9.5	6.3	0.2	0.2	.....	0.2	.....	0.2	379
Gesamtanzahl der Kombination .....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3747

<sup>a</sup> Jede Nummer war meistens durch 2 Pflanzen vertreten.



Züchtern für die Kreuzungen benutzt,—leider sind aber die Methoden dieser Arbeiten in der Literatur nicht bekannt gegeben. Wie Tabelle 9 veranschaulicht, zeigten alle Hybriden  $F_1$  zwischen *Solanum demissum* und den Handelssorten im Freiland keinen Phytophthorabefall. Dasselbe bestätigten auch die Jahre 1932 und 1933. Leider konnten aber in betreff der Ertragsfähigkeit im  $F_1$  während der drei Jahre keine wertvollen Hybriden verzeichnet werden. Die höchste Ertragsfähigkeit wies das Jahr 1934 auf. (Siehe Tab. 9) Im Jahre 1932 betrug der Höchstertrag nur 100 Gramm pro Staude, im Jahre 1933 war er noch bedeutend niedriger. Wie Tabelle zeigt, steht die erhöhte Ertragsfähigkeit offenbar im Zusammenhange mit dem Komponenten aus *S. tuberosum*. So war bei der Kreuzung zwischen *S. demissum* und Alma oder Mirabilis die Ertragsfähigkeit der Hybriden  $F_1$  höher als bei der Kreuzung mit allen übrigen Sorten. Dasselbe Bild zeigten auch die Jahre 1932 und 1933. Der Stärkeprozentsatz war in den meisten Fällen im  $F_1$  der Hybriden höher als bei *S. tuberosum*, wogegen die Form der Knollen bei allen Kombinationen weit hinter derjenigen der Elternsorten aus *S. tuberosum* zurückstand. Dem gesamten Habitus nach stellten diese Hybriden einen Zwischentypus der beiden Arten dar, wobei aber in betreff einiger Merkmale der Blüte und des Blattes das Dominieren von *S. demissum* ziemlich deutlich hervortrat.

Nachstehende Folgerungen konnten gemacht werden:

(1) Die Hybriden zwischen *Solanum demissum*  $\times$  *S. tuberosum* weisen im  $F_1$  eine Reihe von Eigenschaften auf, wie Phytophthoraimmunität, Frostfestigkeit, hohen Stärkegehalt, welche sie wertvoll für die weitere Arbeit machen.

(2) Der Komponent aus *Solanum tuberosum* hat eine wesentliche Bedeutung für den Erfolg der Arbeit und muss einer besonderen Beachtung unterzogen werden.

(3) Zur Erlangung weiterer Generationen der Hybriden ist eine künstliche Bestäubung notwendig.

Im Verlauf der weiteren Arbeit wurde noch die 2te Generation der Hybriden erhalten. Da jedoch die erhaltenen Ergebnisse noch keine Schlussfolgerungen gestatten, sei hier nur kurz erwähnt, dass morphologisch eine sehr starke Aufspaltung beobachtet werden konnte; wie z. B. nach der Färbung der Blüte, der Form und Grösse der Blätter u. s. w. wobei der Habitus der Pflanzen sich stark demjenigen von *Solanum tuberosum* näherte. Im Freiland wurde kein Phytophthorabefall beobachtet.

Grösseres Interesse boten die Hybriden, die durch Rückkreuzungen mit *Solanum tuberosum* im Jahre 1934 erhalten worden waren (Tab. 10). Im Verhalten zum Phytophthorabefall wurde bei diesen Hybriden im Felde eine Aufspaltung beobachtet; der Einfluss von *S. tuberosum* machte sich hier auch geltend, desgleichen auch in Betreff der Ertragsfähigkeit. Wie

die Tabelle zeigt, war die Knollenproduktion einiger Hybriden sogar bei der Vermehrung durch Samen ungefähr gleich derjenigen der Elternformen von *S. tuberosum*. Bei Vermehrung durch Knollen ist sie noch höher. Der Stärkegehalt übertraf bei einer Reihe von Hybriden ebenso wie im  $F_1$  diejenigen der Komponenten aus *S. tuberosum*. Morphologisch machte sich bei diesen Hybriden gleichfalls wie im  $F_2$  eine sehr bunte Aufspaltung bemerkbar, wobei sie ebenso mehr Merkmale von *S. tuberosum* aufweisen, als von *S. demissum*,—was auf den bedeutenden Einfluss des Komponenten aus *S. tuberosum* hinweist. Infolgedessen muss in jedem einzelnen Falle bei der Auswahl dieses Komponenten sein Wert für die Züchtungsarbeit in Betracht gezogen werden.

Bei Rückkreuzungen mit *Solanum demissum* war in der Regel bei langem Tage keine Knollenbildung zu verzeichnen. Morphologisch standen diese Hybriden *S. demissum* nahe, unterscheiden sich jedoch von ihnen durch stärkere Entwicklung der oberirdischen Pflanzenteile und stark ausgeprägte Sterilität, die vollkommen die Möglichkeit des Erhaltens von Beeren ausschloss.

Fassen wir die Ergebnisse dieser Arbeiten zusammen, so können wir feststellen, dass die Kombinierung der Phytophthoraimmunität von *Solanum demissum* mit den wirtschaftlich wertvollen Eigenschaften von *S. tuberosum* möglich ist. Zu diesem Zwecke genügt es aber nicht nur die erste Generation heranzuzüchten, sondern es müssen die weiteren Generationen durch Selbstbestäubung und durch Rückkreuzungen mit *S. tuberosum* erhalten werden. Es können auf diesem Wege sogar Hybriden entstehen, die an Stärkegehalt die Kultursorten, die als Ausgangsformen dienten, überreffen.

Auf Grund des vorhandenen Materials ist voranzusetzen, dass die Aufspaltung der Hybriden, mit den gewünschten Kombinationen von Faktoren, als Resultat von 3–4 wiederholten Rückkreuzungen (*Solanum demissum*  $\times$  *S. tuberosum*) mit *S. tuberosum* zu erwarten wäre.

### III. HYBRIDEN *S. SEMIDEMISSUM* $\times$ *S. TUBEROSUM*

*Solanum semidemissum* stellt laut der Hypothese von Bukasov, eine hybridogene Art dar, entstanden durch die Kreuzung von *S. demissum* mit der Sammelart *S. Antipoviczii*. Diese Art ist von Interesse dank der hier vorhandenen Kombinierung der Phytophthoraimmunität der beiden Arten. Trotzdem die Züchtungsarbeit mit dieser Art infolge ihrer Sterilität sehr erschwert ist, gelang es doch bei der Kreuzung mit *S. tuberosum* "Rotkargis" Hybriden zu erhalten. Inbetreff der Phytophthoraimmunität verhielten sich diese Hybriden im Freiland ebenso, wie die Hybriden der vorhergehenden Gruppe; in Hinsicht der Entwicklung der vegetativen Masse und der Ertragsfähigkeit standen sie aber weit zurück. Der Höchstertrag einer

TABELLE 9.—Ernteertrag und Phytophthoraertrag der  $F_1$  Hybriden von *Solanum demissum* mit *S. tuberosum* und mit *S. andigenum*<sup>a</sup>

Benennung der Ausgangsformen	Phyto- phthorabefall nach dem Vierball- system	Durchschnittsernteertrag pro Staude in Gr.								Prozentsatz des Stärkegehalts						Stärkemenge pro Staude in Gr.					
		Anzahl der Stauden																			
		von 1 bis 50	von 50 bis 100	von 100 bis 150	von 150 bis 200	von 200 bis 250	von 250 bis 300	von 300 bis 350	von 12 bis 14%	von 14 bis 16%	von 16 bis 18%	von 18 bis 20%	von 20 bis 22%	von 22 bis 24%	von 1 bis 20 Gr.	von 20 bis 30 Gr.	von 30 bis 40 Gr.	von 40 bis 50 Gr.	von 50 bis 60 Gr.	von 60 bis 70 Gr.	von 70 bis 80 Gr.
<i>S. demissum</i> × <i>S. tuberosum</i> "Alma"	0 2	2	3	1	1	1	2	1	1	1	4	1	1	3	1	1	2	2	2	1	1
<i>S. demissum</i> × <i>S. tuberosum</i> "Znec"	0 1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>S. demissum</i> × <i>S. tuberosum</i> "Mirabilis"	0 1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>S. demissum</i> × <i>S. tuberosum</i> "Hindenburg"	0 2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>S. demissum</i> × <i>S. tuberosum</i> "Rotkaragis"	0 1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>S. demissum</i> × <i>S. tuberosum</i> "Katahdin"	0 2	1	2	1	4	2	1	1	1	1	1	1	1	2	4	2	2	2	2	1	1
<i>S. demissum</i> × <i>S. tuberosum</i> "Gratiola"	0 2	121	42	25	12	11	3	1	5	6	3	13	10	8	26	10	3	2	2	1	1
<i>S. demissum</i> × <i>S. andigenum</i> f. <i>cryptostylum</i>	0 2	12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>S. demissum</i> × <i>S. andigenum</i> f. <i>huaca laja</i>	0 2	6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

<sup>a</sup> Die Angaben sind bei Feldbeobachtungen in der Nähe von Leningrad im Jahre 1934 erhalten.<sup>b</sup> Der Zähler gibt den Phytophthoraertrag der Hybriden und *S. demissum*, der Nenner den Befall von *S. tuberosum* an.

TABELLE 10.—*Ernteertrag und Phytophthorabefall der Hybride*

Benennung der Eltern- formen:	Anzahl der Sämlinge	Phytophthorabefall nach dem Vierballsystem Anzahl der Stauden <sup>c</sup>					bis 50 Gr.	von 50 bis 100	von 100 bis 150	von 150 bis 200
		0	1	2	3	4				
( <i>S. demissum</i> × <i>S. tuberosum</i> "Alma") × <i>S. tuberosum</i> "Alma" .....	557 <sup>d</sup>	537	9	5	.....	.....	60	52	53	43
( <i>S. demissum</i> × <i>S. tuberosum</i> "Alma") × <i>S. tuberosum</i> "Katahdin" .....	22	20	2	.....	.....	.....	2	3	2	2
( <i>S. demissum</i> × <i>S. tuberosum</i> "Alma") × <i>S. tuberosum</i> "Tannenbergr" .....	35	34	1	.....	.....	.....	7	2	4	7
( <i>S. demissum</i> × <i>S. tuberosum</i> "Alma") × <i>S. tuberosum</i> "Jubel" .....	55	52	2	1	.....	.....	13	8	7	4
( <i>S. demissum</i> × <i>S. tuberosum</i> "Alma") × <i>S. tuberosum</i> "Mirabilis" .....	5	5	.....	.....	.....	.....	.....	.....	.....	.....
( <i>S. demissum</i> × <i>S. tuberosum</i> "Alma") × <i>S. Antipovici- zii</i> × <i>S. tuberosum</i> "Mira- bilis" .....	6	6	.....	.....	.....	.....	2	.....	.....	.....
<i>S. demissum</i> × ( <i>S. demissum</i> × <i>S. tuberosum</i> "Alma") .....	5	5	.....	.....	.....	.....	.....	.....	.....	.....
<i>S. tuberosum</i> "Epicure" × ( <i>S. demissum</i> × <i>S. tube- rosum</i> "Alma") .....	10	10	.....	.....	.....	.....	3	.....	.....	.....
<i>S. tuberosum</i> "Citrus" × ( <i>S. demissum</i> × <i>S. tuberosum</i> "Alma") .....	1	1	.....	.....	.....	.....	.....	.....	.....	.....
( <i>S. demissum</i> × <i>S. tuberosum</i> "Zniec") × <i>S. tuberosum</i> "Zniec" .....	6	6	.....	.....	.....	.....	1	.....	2	2
( <i>S. demissum</i> × <i>S. tuberosum</i> "Mirabilis") × <i>S. tube- rosum</i> "Mirabilis" .....	5	5	.....	.....	.....	.....	4	.....	1	.....
( <i>S. demissum</i> × <i>S. tuberosum</i> "Rotkaragis") × <i>S. tube- rosum</i> "Rotkaragis" .....	145	143	2	.....	.....	.....	41	24	20	9
( <i>S. demissum</i> × <i>S. tuberosum</i> "Rotkaragis") × <i>S. tube- rosum</i> "Katahdin" .....	147	147	.....	.....	.....	.....	43	10	60	9
( <i>S. demissum</i> × <i>S. tuberosum</i> "Rotkaragis") × <i>C. tube- rosum</i> "Hindenburg" .....	100	95	5	.....	.....	.....	13	18	13	9
( <i>S. demissum</i> × <i>S. tuberosum</i> "Rotkaragis") × <i>S. tube- rosum</i> "Jubel" .....	6	6	.....	.....	.....	.....	1	.....	3	.....

*Solanum demissum* × *S. tuberosum* beim Backcross mit *S. tuberosum*<sup>a</sup>

Ernteertrag pro Staude in Gr. <sup>b</sup>															
von 200 bis 250	von 250 bis 300	von 300 bis 350	von 350 bis 400	von 400 bis 450	von 450 bis 500	von 500 bis 550	von 550 bis 600	von 600 bis 650	von 650 bis 700	von 700 bis 750	von 750 bis 800	von 800 bis 850	von 850 bis 900	von 900 bis 950	von 950 bis 1000
d	e	r		S	t	a	u	d	e	n					
28	26	32	18	27	14	11	3	5	8	2	.....	3	.....	.....	1
3	1	2	2	1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
5	1	1	1	1	.....	.....	.....	2	.....	.....	.....	.....	.....	.....	.....
6	7	3	4	1	.....	2	1	.....	.....	.....	.....	.....	.....	.....	.....
.....	3	.....	.....	.....	.....	1	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
1	.....	1	1	2	.....	.....	1	1	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
15	8	7	.....	8	3	.....	1	.....	.....	.....	.....	.....	.....	.....	.....
3	2	3	3	1	1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
11	10	11	6	2	4	.....	1	1	.....	1	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

<sup>a</sup> Die Angaben sind im Jahre 1934 beim Feldanbau erhalten.

<sup>b</sup> Der Ernteertrag der verschiedenen Sorten von *S. tuberosum* betrug pro Staude von 300 bis 1400 Gramm.

<sup>c</sup> Den Phytophthorabefall der verschiedenen Sorten von *S. tuberosum*—Tabelle 9.

<sup>d</sup> Sämlinge sind ausgefallen.

Staude betrug während der ganzen Zeit nur 40 Gr. Die Sterilität dieser Hybriden machte die weitere Arbeit mit diesen Hybriden fast zur Unmöglichkeit.

TABELLE 11.—*Ernteertrag und Phytophthorabefall der F<sub>1</sub> Hybriden Solanum Antipoviczii × S. tuberosum*<sup>a</sup>

Benennung der Elternformen	Anzahl der Sämlinge	Phytophthorabefall nach dem Vierball- system					Durchschnittsertrag pro Staude in Gr.				
		0	1	2	3	4	50 bis 100	100 bis 150	150 bis 200	200 bis 250	
		Anzahl der Sämlinge					von — bis	von 50 bis	von 100 bis	von 150 bis	von 200 bis
<i>S. tuberosum</i> "Mirabilis"											
× <i>S. Antipoviczii</i> <sup>b</sup> .....	1	1	..... <sup>c</sup>	.....	.....	.....	.....	.....	1	.....	.....
<i>S. tuberosum</i> "Epikure"											
× <i>S. Antipoviczii</i> .....	2	2	.....	.....	..... <sup>c</sup>	.....	.....	1	1	.....	.....
<i>S. tuberosum</i> "Imperator" (Richter)											
× <i>S. Antipoviczii</i> .....	2	2	.....	.....	.....	..... <sup>c</sup>	1	1	.....	.....	.....

<sup>a</sup> Die Angaben sind im Jahre 1934 beim Feldanbau erhalten.

<sup>b</sup> Der Ernteertrag der verschiedenen Sorten von *S. tuberosum* betrug von 900 bis 1200 gr. pro Staude.

<sup>c</sup> Phytophthorabefall *S. tuberosum*.

#### IV. HYBRIDEN *S. ANTIPOVICZII* × *S. TUBEROSUM*

*Solanum Antipoviczii* wurde im Jahre 1928 zum erstenmal als. Art von Bukasov beschrieben; ihr charakteristischer Unterschied von *S. demissum* besteht für die Züchtungsarbeit darin, dass sie sich nur sehr schwer mit *S. tuberosum* kreuzen lässt. Erst nach andauernder Arbeit mit dieser Art wurde bei Kreuzungen mit Handelssorten eine Reihe von Hybriden erhalten, wobei diese Art sowohl als Mutterpflanze, wie als Vaterpflanze benutzt wurde (Tabelle 11). Die Generation aller drei Kombinationen stand morphologisch (dem Gesamthabitus, der Stengel- und Blätterfärbung, dem Bau der Blätter und Blüten nach) näher zu *S. Antipoviczii* als zu *S. tuberosum*. Besonders deutlich trat es bei den beiden letzten Kombinationen hervor. Alle Hybriden waren widerstandsfähig gegen *Phytophthora infestans*. Die Ertragsfähigkeit stand bedeutend hinter derjenigen der besten F<sub>1</sub> Hybriden von *S. demissum* × *S. tuberosum* zurück. Die Tabelle zeigt den Höchstertrag während einer dreijährigen Arbeit mit der ersten Kombination und einer zweijährigen mit der II und III Kombination der Hybriden. Da die erste Generation dieser Hybriden bloss als Ausgangsmaterial für die

weiteren Kreuzungen dienen kann, muss die Frage der Beerenbildung besonders in Betracht gezogen werden. Die Kombination *S. Antipoviczii* × *S. tuberosum* Mirabilis bildete im  $F_1$  bei natürlicher und bei künstlicher Selbstbestäubung sowie bei der Kreuzung mit *S. tuberosum* sehr leicht Beeren. Bei beiden anderen Kombinationen konnte eine Beerenbildung nur mit grosser Mühe erzielt werden, was auf den Einfluss des mütterlichen Elters aus *S. tuberosum* zurückzuführen ist. Die weitere Arbeit beschränkte sich nur auf die erste Kombination. Wie aus der Tabelle 12 zu ersehen ist, waren alle  $F_2$  Hybriden und  $F_1$  von Rückkreuzungen beim Befall im Freiland immun, während *S. tuberosum* je nach der Sorte eine verschiedene Stärke des Befalles aufwies. (Epikur erhielt die Nummer 4, Mirabilis und Jubel—3.) Der Stärkegehalt übertraf bei einer Anzahl von Hybriden denjenigen der Ausgangsformen aus *S. tuberosum*, da jedoch diese Angaben bloss das Ergebnis der Arbeit eines Jahres wiedergeben, und zwar bei der Heranzüchtung der Kartoffel aus Samen,—haben sie nur einen relativen Wert. Zu den unerwünschten Eigenschaften einer neuerzüchteten Sorte, die diese Hybriden jedoch aufwiesen, gehören die langen Stolonen, die Spätreife und die dunkle Färbung der Knollen. Die beiden ersten Eigenschaften weisen auf den grossen Einfluss von *S. Antipoviczii* im  $F_2$  und bei Rückkreuzungen hin. Der Einfluss dieser Art, die einen recht scharf ausgeprägten homozygoten Typus darstellt, macht sich auch in der morphologischen Einheitlichkeit der Hybriden besonders in den 3 ersten Kombinationen bemerkbar. Bei den Hybriden Epikur × (*S. Antipoviczii* × *Mirabilis*) treten die Eigenschaften von *S. Antipoviczii* weniger scharf hervor. Trotz der verhältnismässig geringen Anzahl dieser Hybriden kann man dennoch nach den Angaben des Jahres 1934, recht gut morphologisch und physiologisch die bunte Aufspaltung beobachten.

In Betreff dieser Hybriden kann kein endgültiger Schluss gezogen werden; es kann nur festgestellt werden, dass die vorhandenen Hybriden als Ausgangsmaterial benutzt werden können; ob aber unter diesen Hybriden sich der Grundleger einer neuen Sorte auffinden lässt, ist noch nicht klar. Die Sterilität bestimmt das Schicksal und den Wert der Hybriden. In dieser Hinsicht unterscheiden sich die in der Tabelle 12 angeführten Kombinationen sehr stark voneinander. Die  $F_2$  Hybriden hatten ungefähr 70% Sämlinge mit guter Beerenbildung, während die Hybriden von Rückkreuzungen weder bei natürlicher noch bei künstlicher Bestäubung im Jahre 1934 Beeren ansetzten. Die mikroskopische Analyse des Pollens einiger Pflanzen dieser Hybriden stellte seine Verkümmerteit fest. Dieses weist einerseits auf eine anormale Reduktionsteilung hin, anderseits zeigt diese Tatsache dem Züchter die Schwierigkeiten, auf die er bei der Anwendung dieser Methode stossen wird.

TABELLE 12.—Ernteertrag und Phytophthorabefall der  $F_2$  hybriden *S. Antipo*

Benennung der Elternformen	Anzahl der Samlinge	Phytophthorabefall nach dem Vierballsystem					von bis 50 gr.	von 50 bis 100	von 100 bis 150	von 150 bis 200	von 200 bis 250
		0	1	2	3	4					
$F_2$ <i>S. Antipoviczii</i> × <i>S. tuberosum</i> "Mirabilis" .....	127	157	..... <sup>b</sup>	.....	.....	.....	21	12	22	14	10
( <i>S. Antipoviczii</i> × <i>S. tuberosum</i> "Mirabilis") <sup>b</sup> × <i>S. tuberosum</i> "Mirabilis" .....	598	598	.....	.....	.....	.....	5	17	26	33	43
( <i>S. Antipoviczii</i> × <i>S. tuberosum</i> "Mirabilis") × <i>S. tuberosum</i> "Jubel" .....	12	12	.....	..... <sup>b</sup>	.....	.....	.....	.....	.....	.....	.....
<i>S. tuberosum</i> "Epikur" × ( <i>S. Antipoviczii</i> × <i>S. tuberosum</i> "Mirabilis") .....	9	9	.....	.....	.....	..... <sup>b</sup>	.....	.....	.....	.....	.....

<sup>a</sup> Die Angaben sind beim Feldanbau im Jahre 1934 erhalten.

<sup>b</sup> Der Phytophthorabefall von *S. tuberosum*.

<sup>c</sup> Ernteertrag pro Staude von *S. tuberosum* von 500 bis 1000 gr.

Zusammenfassend kann man vorläufig in Betreff der Hybriden dieser Art nur feststellen:

(1) Bei Benutzung dieser Art zur Heranzüchtung fester Sorten müssen durch Selbstbestäubung und Rückkreuzungen weitere Generationen herangezüchtet werden.

(2) Die durch Selbstbestäubung erhaltenen Hybriden sind in der Regel weniger ertragsreich als diejenigen von Rückkreuzungen.

(3) Die Eigenart von *S. Antipoviczii* ist im  $F_2$  der beiderlei Hybriden schärfer ausgeprägt als bei analogen Kreuzungen zwischen *S. demissum* und *S. tuberosum*.

(4) Kreuzungen zwischen *S. Antipoviczii* und *S. tuberosum* gelangen viel schlechter als Kreuzungen zwischen *S. demissum* und *S. tuberosum*—was eine weitere systematische Entfernung dieser Art von *S. tuberosum* vermuten lässt.

(5) Die Auswahl des Elters aus *S. tuberosum* hat bei dieser Kreuzung eine sehr grosse Bedeutung.

(6) Die wirtschaftlich wertvollen Eigenschaften, die sich an dem vorhandenen Material beobachten liessen, weisen auf die Möglichkeit hin in den weiteren Generationen aus Rückkreuzungen Hybriden zu erzüchten, die den an die Kartoffel gestellten Forderungen gerecht werden können.

Leider aber wird die Heranzüchtung immuner Kartoffelsorten noch von dem Vorhandensein biologischer Rassen des Parasiten (auf die zuerst im



iczi mit *S. tuberosum* und deren  $F_2$  hybriden beim backcross mit *S. tuberosum*<sup>a</sup>

Ernteertrag pro Staude in Gr.<sup>c</sup>

von 250 bis 300	von 300 bis 350	von 350 bis 400	von 400 bis 450	von 450 bis 500	von 500 bis 550	von 550 bis 600	von 600 bis 650	von 650 bis 700	von 700 bis 750	von 750 bis 800	von 800 bis 850	von 850 bis 900	von 900 bis 950	von 950 bis 1000	Mehr als 1000
Anzahl der Stauden															
7	5	8	10	3	6	.....	2	2	.....	.....	.....	.....	1	.....	.....
60	59	64	53	44	54	26	33	18	20	6	9	2	7	.....	2
1	2	.....	1	3	1	.....	.....	1	.....	.....	.....	.....	.....	.....	.....
1	1	.....	.....	.....	1	.....	1	1	1	1	1	.....	.....	1	.....

Jahr 1932 von Schick (8) hingewiesen wurde) sehr erschwert. Die gegen einen Biotyp des Parasiten herangezüchteten immunen Kartoffeln können von einem anderen Typ befallen werden, wie es in Deutschland (Müller (6)) nach der Mitteilung von Schick (8) geschehen ist. Es erhebt daher die Notwendigkeit das Ausgangsmaterial in Betreff verschiedener Herkünfte der *Phytophthora infestans* zu prüfen um den Züchter vor unerwarteten Misserfolgen zu schützen.

## SUMMARY

Die Kartoffelzüchtung, die sich zum Ziel das Heranzüchten von phytophthorawiderstandsfähigen Sorten stellt, hat diese Aufgabe trotz langjähriger Arbeit noch nicht lösen können. Der Grund hierfür liegt offenbar in der ungenügenden Kenntnis des nötigen Ausgangsmaterials.

Im Verlauf der letzten 10 Jahre hat das Institut für Pflanzenbau in UdSSR dank seinen Expeditionen eine grosse Weltkollektion von Kartoffeln angelegt, und die grosse Mannigfaltigkeit dieses Materials von Arten und Formen systematisiert. Dieses gestattet gegenwärtig der Frage nach dem Ausgangsmaterial mit mehr Kenntnissen näher zu treten, und bis zu einem bestimmten Grad die Zufälligkeit bei der Wahl der beiden Kreuzungskomponenten auszuschliessen. Unter den endemischen Sorten der Kulturkartoffeln aus Süd- und Central Amerika sind gegenwärtig bei der polymorphen Art *S. andigenum* hochresistente Formen und Varietäten festgestellt wor-

den. In dieser Hinsicht sind von besonderem Interesse die Länder Kolumbien, Bolivien und Mexiko. Alle die übrigen von uns untersuchten endemischen südamerikanischen Kulturarten der Kartoffel wurden gewöhnlich ziemlich stark von der *Phytophthora* befallen.

Als Lokalisierungsort der phytophthoraimmunen Arten, die unter den wilden Kartoffelarten aufgefunden wurden, kann Mexiko gezählt werden. Für dieses Land können 9 phytophthoraimmune wilde Kartoffelarten angegeben werden. Systematisch stehen diese Arten verschieden weit von den Kulturkartoffeln ab; infolgedessen weisen diese Arten für die Ausnutzung als Züchtungsmaterial auch verschiedenen Wert auf.

Es ist vorauszusetzen, dass das Heranzüchten phytophthoraimmuner Kartoffeln (1) durch Kreuzung zwischen der Kulturart *S. andigenum* und Handelssorten und (2) durch Kreuzung zwischen wilden Arten und Handelssorten erreicht werden kann. Die Kreuzungen von *S. andigenum* mit *S. tuberosum*, ebenso wie die Sortenkreuzungen von *S. tuberosum* gestatteten schon im  $F_1$  phytophthorawiderstandsfähige Sorten zu erhalten, die zugleich auch eine gute Ertragsfähigkeit und hohen Prozentgehalt von Stärke aufwiesen. Dennoch ist für die Heranzüchtung einer phytophthoraimmunen Sorte hier wenig Wahrscheinlichkeit vorhanden. Bei Kreuzungen zwischen *S. demissum* und *S. tuberosum* können gute Ergebnisse nur in den weiteren Generationen (im  $F_2$ ,  $F_3$  u. s. w.) erwartet werden. Besondere Aufmerksamkeit verdienen die Rückkreuzungen, da sie voraussichtlich die Möglichkeit geben werden am schnellsten in der neu erzüchteten Sorte die Vereinigung der Phytophthoraresistenz mit anderen wirtschaftlich wertvollen Eigenschaften zu erlangen. Bei Anwendung dieser Methode sind die gewünschten Resultate nach 3–4 Rückkreuzungen zu erwarten.

Im wesentlichen müssen die Kreuzungen zwischen *S. Antipoviczii* und *S. tuberosum* nach derselben Methode durchgeführt werden, wie mit *S. demissum*. Als Unterschied bei diesen Kreuzungen wäre in den Hybriden dieser Gruppe die schärfere Ausgeprägtheit der Eigentümlichkeit der wilden Arten nicht nur im  $F_1$ , sondern auch im  $F_2$  und in den Hybriden der Rückkreuzungen zu erwähnen.

Die Phytophthorawiderstandsfähigkeit, die Ertragsfähigkeit und der Stärkegehalt ist bei den  $F_1$  Hybriden der Rückkreuzungen *S. Antipoviczii*  $\times$  *S. tuberosum* mit *S. tuberosum* höher, als bei den auf die gleichen Weise erhaltenen Hybriden von *S. demissum*. Leider sind aber die oben angeführten Hybriden sehr steril, wodurch die Arbeit mit ihnen stark erschwert wird.

Bei allen Kreuzungen der wilden Arten mit *S. tuberosum* muss das  $F_1$  im Gegensatz zu den Kreuzungen der Kultursorten nur als Ausgangsmaterial für die weiteren Züchtungsarbeiten angesehen werden.

Die Erlangung von phytophthorafester Sorten scheint aber auch möglich zu sein durch Heranziehung anfälliger wilder Arten zu der Kreuzung mit *S. tuberosum*. Dieses findet seine Erklärung in der Möglichkeit des Zustandekommens von Kombinationen, bei denen sich die Komplexe der die Immunität bedingenden Gene dieser beiden Arten in den Hybriden gegenseitig ergänzen.

Die Arbeit der Immunitätszüchtungen gegen die Kartoffelfäule gestaltet sich in der letzten Zeit noch schwieriger durch die Feststellung von biologischen Rassen des Parasiten, die sich durch ihre Virulenz von einander unterscheiden. Es ist jedoch zu hoffen, dass das Heranziehen zu den Züchtungsarbeiten von wilden und Kulturarten der Kartoffel es ermöglichen wird, den Genenbestand der Kultursorten in Betreff der Immunität zu bereichern, und dadurch die Erzüchtung von Sorten, die gegen alle Biotypen widerstandsfähig wären, möglich zu machen.

#### SUMMARY

Breeders of Irish potatoes whose aim is the production of varieties resistant to Phytophthora have not yet solved their problem, in spite of many years of work. The cause apparently lies in the insufficient knowledge of the necessary stock material with which to work.

Thanks to various large expeditions, the Institute of Plant Breeding in U.S.S.R. during the last 10 years has made a large world collection of Irish potatoes, and has now grouped this material, rich in species and forms. This collection now permits a more intelligent consideration of the question of the breeding stock, and thus the exclusion to a certain degree of any random in the selection of any 2 components for crossing. Of the endemic varieties of cultivated potatoes of South and Central America, highly resistant forms and varieties were observed in the polymorphic species *Solanum andigenum*. In this regard Colombia, Bolivia, and Mexico are of especial interest. All the remaining endemic South American cultural species of potatoes investigated by us were, as a rule, quite heavily attacked by Phytophthora.

Mexico can be counted as the geographical center of the Phytophthora-immune species found from among the wild potato species. To this country there can be credited 9 phytophthora-immune wild potato species. Taxonomically, these species stand at quite different distances from the cultivated potatoes, and, accordingly, their value as breeding stock varies greatly.

It is to be presumed that the breeding of potatoes immune from Phytophthora may be successful, (1) through crossing between the cultivated species *Solanum andigenum* and commercial varieties, and (2) through crossing between wild species and commercial varieties. The hybrids of *S. andigenum* and *S. tuberosum*, as well as the varietal crossings of *S. tubero-*

*sum*, may lead in the  $F_1$  generation to varieties resistant to *Phytophthora*—varieties that in addition show good productiveness and a high percentage of starch. In spite of this, there is little possibility here for the breeding of a variety immune from *Phytophthora*. In crossings between *S. demissum* and *S. tuberosum* good results could be expected only in later generations ( $F_2$ ,  $F_3$ , etc.). Backcrossings deserve special consideration, because they might produce most quickly varieties that combine resistance to *Phytophthora* with other economically valuable characters. In the application of this method the desired results may be expected after 3 to 4 backcrossings.

The crossings between *Solanum Antipoviczii* and *S. tuberosum* should, in the main, follow the same method as with *S. demissum*. As a distinctive feature resulting from crossings in this group there might be mentioned the stronger accentuation of the characters of the wild species in the ensuing hybrids, not only in  $F_1$ , but also in  $F_2$  and in the hybrids of the backcrosses.

The resistance to *Phytophthora*, the productiveness, and the starch content are higher in the  $F_1$  hybrids of the backcrosses *S. Antipoviczii*  $\times$  *S. tuberosum* with *S. tuberosum* than in the hybrids of *S. demissum* similarly bred. The aforementioned hybrids are unfortunately sterile, a fact that hampers the work with them considerably.

In all crosses of wild species with *S. tuberosum*, as opposed to those of cultural varieties,  $F_1$  must be considered only as stock material for further breeding work.

It seems possible that varieties resistant to *Phytophthora* may also be obtained by the use of susceptible wild species in the hybridization with *S. tuberosum*. The explanation for this lies in the possibility of combinations in which the gene complexes conditioning immunity in the 2 species may supplement each other in the hybrids.

Of late the work of the breeder for immunity from potato blight has become still more difficult through the identification of biological races in the parasite that differ in their virulence. It is to be hoped that in breeding work the use of wild and cultural species of the potato will make it possible to enrich the gene complex for immunity in the cultural varieties and thereby make possible the breeding of varieties resistant against all biotypes of the parasite.

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# CERTAIN VIROSES OF THE GARDEN PEA, *PISUM SATIVUM*

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## INTRODUCTION

Numerous records of viroses of the garden pea, *Pisum sativum* L., have been made. The various types of symptom expression reported and the conflicting evidence as to host range suggest that a number of distinct viruses affect this host. It was the purpose of the investigation here reported to study symptoms, host ranges, physical properties, and modes of transmission of certain viruses that affect garden pea.

The first record of pea mosaic known to the writer is that made by Dickson (4) in Canada in 1920. Numerous reports of the occurrence of mosaic and "mosaic-like" diseases of peas followed. By 1928, such reports had come from California,<sup>2</sup> New Jersey, Maryland, Wisconsin, Utah, Indiana, Minnesota, Idaho, Wyoming, Montana,<sup>3</sup> Michigan,<sup>4</sup> and Washington.<sup>5</sup>

Doolittle and Jones (7) seem to have been the first to report transmission of a pea-mosaic virus by mechanical means and by the pea aphid, *Macrosiphum pisi* Kalt., although it is possible that Taubenhause (27) dealt with the same virus in sweet pea, *Lathyrus odoratus* L. Later, Zaumeyer (31) and Zaumeyer and Wade (32, 33, 35) transmitted pea mosaic by expressed juice, while they and others (15, 18, 24) have been successful in transmitting pea mosaic by means of the pea aphid. Osborn (17, 18) distinguished two mosaic viruses of pea, both of which were transmitted by the potato aphid, *Macrosiphum gei* Koch, and by the pea aphid. One of the viruses required an incubation period in both species of aphid and was carried by the aphids throughout their lives, while the second virus did not require an

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<sup>2</sup> Vaughan, R. E. Diseases of field and vegetable crops in the United States in 1923. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Repr., Sup. 34: 148-243. 1924. [Mimeographed.]

<sup>3</sup> Linford, M. B. Pea diseases in the United States in 1928. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Repr., Sup. 67. 1929. [Mimeographed.]

<sup>4</sup> Jehle, R. A. and J. I. Wood. Diseases of field and vegetable crops in the United States in 1925. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Repr., Sup. 45. 1926. [Mimeographed.]

<sup>5</sup> Haskell, R. J. and J. I. Wood. Diseases of vegetable and field crops other than cereals in the United States in 1926. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Repr., Sup. 54. 1927. [Mimeographed.]

incubation period, and aphids carrying it soon lost their ability to infect healthy plants.

Various types of mosaic symptoms on peas have been observed by Linford,<sup>6</sup> and Merkel (15). These consist primarily of variations in mottle patterns on foliage. Böning (1) also observed two distinct types of mosaic symptoms on broad bean (*Vicia faba* L.) infected with a virus that he believed to be identical with a pea-mosaic virus. Johnson and Jones (10) and Zaumeyer and Wade (33, 35) recognize more than one mosaic disease of peas. In addition to the diverse types of foliage mottling reported, there are other differences in symptoms, such as the degree of stunting of the plants, amount of pod and foliage distortion, and the production of enations on the leaves. Snyder (24) has emphasized the importance of pod malformation of peas grown in California. One of the viruses distinguished by Osborn (18) caused wrinkling of the leaves, some stunting of the plants, yellowish (and later white) spots on the leaves. *Pea virus 3*, as described by Pierce (21), produces mottling of pea foliage.

The transmission of pea-mosaic viruses to or from other leguminous hosts has been reported by various workers. Böning (1) worked with a virus that infected pea, red clover, *Trifolium pratense* L., crimson clover, *T. incarnatum* L., and broad bean. Merkel (15) succeeded in cross-inoculating many legumes with legume-mosaic diseases when he used different species of aphids. The pea aphid transmitted pea mosaic to sweet pea, red clover, garden bean, *Phaseolus vulgaris* L., alfalfa, *Medicago sativa* L., and lupine, *Lupinus* sp. He also infected peas with viruses from sweet pea, red clover, and lupine. Chamberlain (3) reported that pea mosaic is a virus disease common on garden peas throughout New Zealand and that the same virus also attacks sweet peas, broad bean, lupins, red clover and other clovers. Similarly, Doolittle and Jones (7) found the mosaic diseases of garden pea, sweet pea, and red clover to be intertransmissible. It is also of interest to note that Osborn (18) worked with a mosaic virus from garden pea that infected broad bean, sweet pea, and crimson clover, but not red clover. Other conflicting conclusions on the host range of legume-mosaic viruses affecting pea developed when Zaumeyer and Wade (32, 33, 35) found that the red-clover-mosaic virus, inoculated on the pea, produced symptoms somewhat similar to pea mosaic. They also worked with a mosaic virus from pea that infected 31 out of 32 varieties of beans tested; many plants of the very susceptible bean varieties were killed by this mosaic. Other hosts infected by this virus were sweet pea, white sweet clover, white clover, broad bean, and a few other legumes including several species of *Phaseolus*. Peas and sweet pea were not infected by the common bean-mosaic virus. However, Pierce (20) secured infection on pea (Perfection variety) by inoculation with *alfalfa virus 2*, and later (21) demonstrated that *bean virus 2* infects pea.

<sup>6</sup> See footnote 3, p. 242.

A streak disease, believed to be caused by a virus, was observed by Linford on peas in various parts of the United States, in 1928.<sup>7</sup> This disease was characterized by necrosis of pods and of the phloem of stems and leaves. Occasionally, necrosis of the entire upper part of the plant occurred. Later, Linford (14) produced similar symptoms on peas in Hawaii as a result of the feeding of thrips, *Thrips tabaci* Lindeman, transferred from *Emilia sagittata* (Vahl.) DC. infected with the pineapple yellow-spot virus. He found streak caused by this virus in market-garden plantings of peas near Honolulu, and suggested that pea streak, in the United States, is caused by the latter virus or by a related one. It is of interest in this connection that Whipple (29) has found the virus of spotted wilt of tomato to be a causal factor in pea streak also. Zaumeyer and Wade (32) also observed a necrotic streak on pea and sweet pea inoculated with viruses from sweet clover, *Melilotus alba* L., and white clover, *Trifolium repens* L., and they obtained a similar result later with a virus from alfalfa (34).

Seed transmission of pea mosaic was reported by Dickson (5, 6). He observed plants from several lots of seed and found infected seedlings ranging from 5 per cent to 76.3 per cent. Merkel (15) and Zaumeyer and Wade (32, 33, 35) report evidence of occasional infected seedlings. On the other hand, Doolittle and Jones (7) found no evidence of seed-transmission of the pea-mosaic virus with which they worked, although they grew and observed over 1900 seedlings from seed collected from infected plants. Van der Meulen (16) in a more limited trial also failed to secure transmission through the seed.

#### MATERIALS AND METHODS

##### Sources of Viruses Studied

Three mosaic pea plants, found in experiments on seed transmission, displayed 3 distinct types of symptoms. The infected plants were all of the variety Mammoth Melting Sugar and of the same age. Transfer of aphids, *Macrosiphum pisi*, from each diseased plant to healthy seedlings of the same variety and other varieties resulted in production of the 3 symptom types, without intermediate gradations. Continued transfer from this infected material gave further evidence that the symptom differences were due to virus differences rather than variation in the host material and environmental conditions. These 3 viruses will be shown to be practically identical in their physical properties, mode of transmission, and host range. They differ only in the symptoms produced, and are considered as strains of the same virus. The diseases and the virus strains are designated as follows:<sup>8</sup>

<sup>7</sup> See footnote 3, p. 242.

<sup>8</sup> While this manuscript was in process of publication, other contributors used these numbers to designate pea viruses; but, since their use of the numbers was for convenience rather than actual designation of a described virus, and, because in other instances the viruses were later found to be similar to viruses having a recognized designation, the virus designations here used were not changed. The pea mosaic viruses described here are discussed in relation to other legume viruses.



*Marble Pea Mosaic* (*pea virus 2A*); *Speckle Pea Mosaic* (*pea virus 2B*); *Mild Pea Mosaic* (*pea virus 2C*). During studies with the 3 strains of the virus listed above it was found that none of them would infect the Perfection variety of peas. Since a mosaic disease of this variety had been seen many times by the author and reported by Snyder (24), it was believed that still another pea-mosaic virus could be found. Perfection peas grown in a plot at Madison, Wisconsin, in 1934, supplied such a virus, which appeared to be the same as that studied by Snyder (24) and one of those used by Osborn (18). This disease will be referred to as *Enation Pea Mosaic* and the virus designated as *pea virus 1*.

Authentic tobacco ring spot was obtained from the supply maintained by B. M. Duggar and his associates. The virus was maintained on tobacco, as it had been previously. Pierce (20) reported negative results when attempts were made to inoculate Perfection peas with this virus by rubbing expressed juice on the foliage. Two other methods of applying inoculum, to be described later, successfully transferred the *tobacco ring-spot virus* to peas during the studies herein reported.

### Methods

Field trials were conducted on the Experiment Station farm at Madison, Wisconsin, during the summers of 1933 and 1934. The plot chosen was surrounded by a legume nursery containing many varieties and strains of alfalfa and sweet clover, and a few volunteer plants of red and alsike clover. A high percentage of these legumes appeared to be affected with viroses. In 1932 a similarly located pea plot was badly diseased with what was believed to be one or more of these diseases.

Studies on varietal susceptibility, seed transmission, insect and mechanical transmission, and virus properties were conducted in a greenhouse in which the average daily temperature was about 20–22° C. Frequent fumigation was practiced to keep the house free from aphids. Seedling peas, about 14 days from seed, were used in nearly all of the studies. Other host plants were inoculated in an early stage of their growth.

Doolittle and Jones (7) secured mosaic infection on peas by rubbing the foliage with expressed juice from diseased plants and by inserting macerated diseased tissue into a slit at the base of the stem. Merkel (15) transmitted pea mosaic to sweet pea and broad bean by inserting macerated diseased tissue into a wound made on healthy plants. Osborn (18) applied expressed juice from diseased plants by rubbing and by pricking with needles, but obtained very little infection. Attempts were made to transmit the 3 strains of *pea virus 2* by these methods, but very few infections resulted. Further tests showed that insertion of infected tissue into the stem of healthy seedlings was the effective part of these methods of inoculation as far as the trans-

mission of strains of this pea virus was concerned. The infected tissue was macerated as it was pushed into the seedling stem. No infections were obtained when expressed juice was applied by rubbing with cheesecloth or when the juices were pricked or injected into the stems. Still further trials showed that transmission by inserting infected tissue into the stem was most effective when the insertion was made between the two scale leaves or between the scale leaves and the cotyledonary node. This method of applying inoculum also proved effective in infecting pea seedlings with *pea virus 1* and the *tobacco ring-spot virus*. Pea varietal reactions were determined with this method of inoculation.

Osborn (18) used "norit" with expressed juice without appreciable success. The method of Rawlins and Tompkins (23) in which carborundum was used as an abrasive with expressed juice was found to be a successful means of obtaining infection with the pea viruses used. This procedure was followed in making property and host-range studies of the viruses. Inocu-

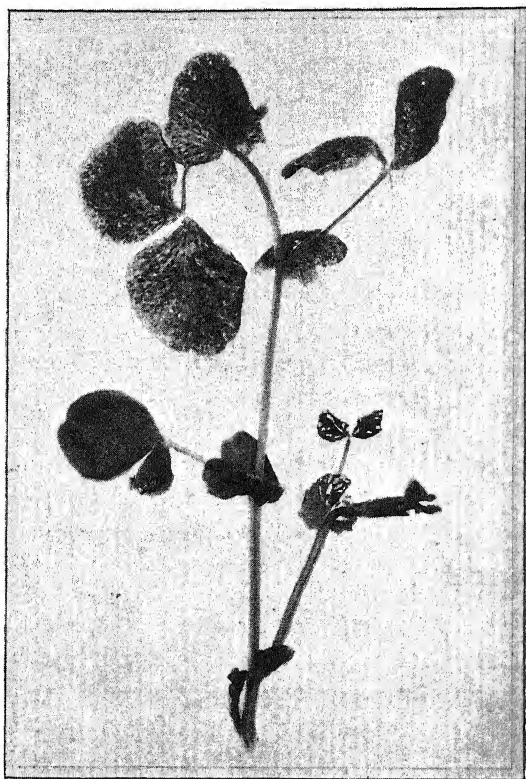


FIG. 1. Alderman seedling infected with *pea virus 1* showing pronounced vein-clearing, distortion of top foliage, and transparent spots on secondary stem foliage.

lum was prepared for these studies by crushing leaves and stems of infected plants in a sterile mortar and straining the juice through cheesecloth.

Aphid transmission experiments with *Macrosiphum pisi* were conducted by transferring aphids grown on healthy plants to infected plants, where they were allowed to feed for 36 to 48 hours. At the end of this feeding period 10 to 15 aphids were transferred to each healthy plant to be infected. Transfer of infected foliage bearing a colony of aphids was used most frequently. Another feeding period of 36 to 48 hours was provided before the aphids were killed by fumigation with nicotine dust. Lantern globes or 30-mesh wire cages were used to keep the aphids confined to the test plants.

#### SYMPTOM EXPRESSION

Differences in symptoms among the diseases were used early in the studies as a basis for distinguishing the different viruses. In general, the symptoms of these diseases were found to be most typical on Alderman peas, although a few outstanding departures from the typical symptoms were observed. The following descriptions applied to the diseases as they developed on Alderman seedlings at 20–22° C., except where other conditions are stated.

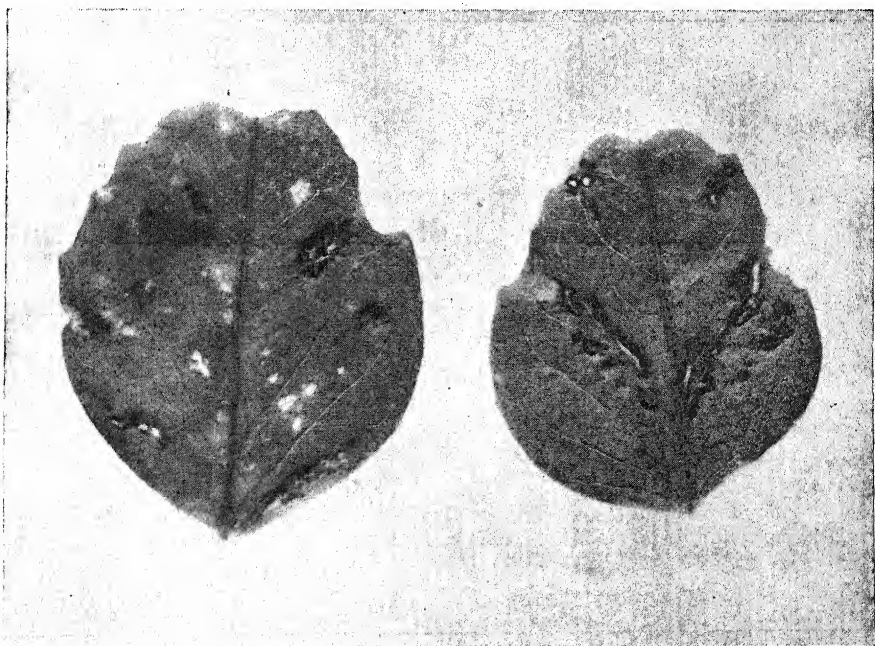


FIG. 2. Lower surface of pea leaflets showing light spots and leaf enations from infection by *Pea virus 1*.

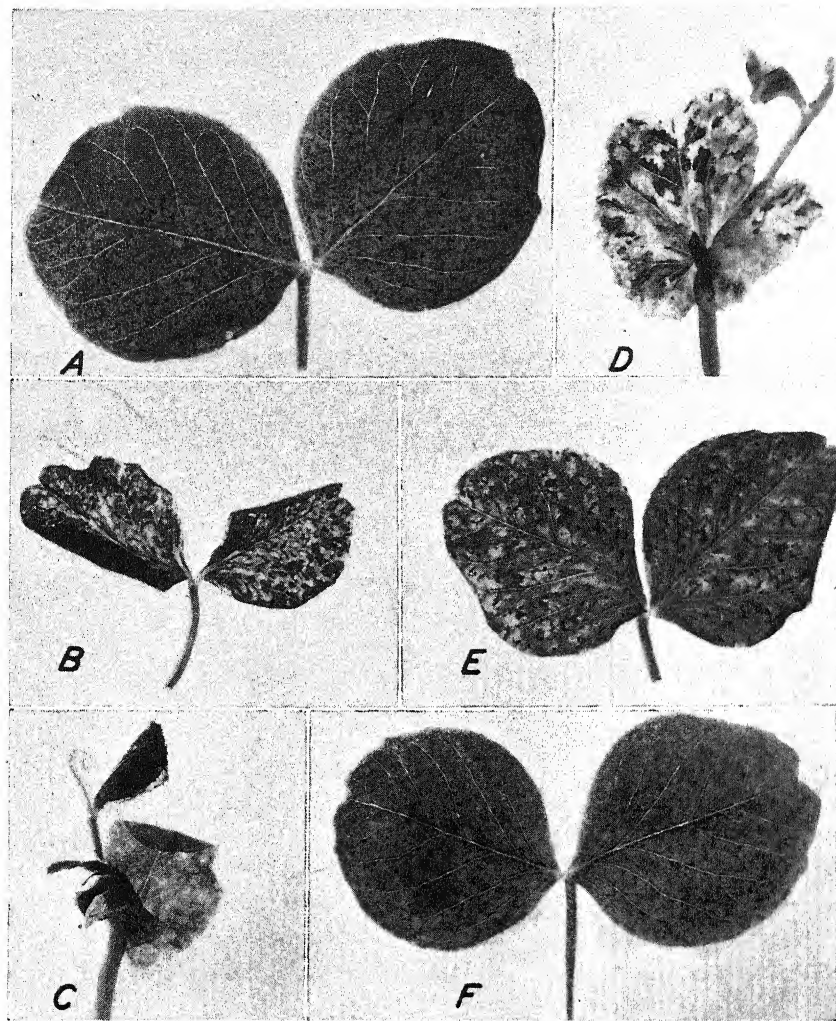


FIG. 3. Alderman leaflets and stipules showing symptoms produced by virus infection. A. Leaflets from noninoculated plant. B. Leaflets infected with *pea virus 1*. C. Necrosis of leaflets, stipules and terminal bud resulting from infection with *tobacco ring-spot virus*. D. Stipules and leaflets from plant infected with *pea virus 2A*. E. Leaflets infected with *pea virus 2B*. F. Mild mosaic on leaflets infected with *pea virus 2C*.

#### Enation Pea Mosaic (*pea virus 1*)

In seedlings inoculated with *pea virus 1*, symptoms developed on the 6th or 7th day. The first symptom to appear was pronounced vein clearing in the most recently developed leaflets and stipules (Fig. 1 and Fig. 3, B).

Continued enlargement and fusion of chlorotic areas usually left the first symptom-bearing tissue nearly devoid of any color-producing pigments. Such tissue remained turgid for a few days, but then became flaccid and gray. At this stage of disease development the buds and all foliage about them were much distorted and almost invariably turned downward or twisted to one side (Fig. 1). Retardation of terminal growth appeared to stimulate axillary bud development in most cases. Foliage produced on secondary stems was distorted, very much reduced in size, and bore small circular or elongated transparent spots (Fig. 1). No mottle symptom was expressed on this foliage. Examination of the lower side of leaflets and stipules showed that each transparent spot was bordered by a ridge of proliferated tissue that appeared to be an enation somewhat like that described by Jensen (9). Many other such ridges occurred along veins without apparent relationships to chlorotic areas (Fig. 2). None of the other viruses studied ever produced such a symptom on field or greenhouse-grown plants.

Older plants infected with *pea virus 1* did not exhibit the marked bud and foliage distortion that occurred on seedlings. Foliage symptoms were limited to wrinkling, some reduction in size, and numerous chlorotic to transparent spots with their accompanying enations. Observation of greenhouse- and field-grown plants bearing pods revealed that this virus also produced pod malformation like that described by Snyder (24). *Pea virus 1* was the only virus studied that produced distortion of pods.

Dwarf Telephone seedlings developed severe top necrosis when inoculated with *pea virus 1*. Many Horsford seedlings were killed completely.

#### Marble Pea Mosaic (*pea virus 2A*)

When plants were inoculated with this virus by any of the methods of inoculation previously described, the first symptom to appear was vein clearing in the newest leaflets and stipules produced during the 6- to 8-day incubation period. The next set of leaflets and stipules produced were much reduced in size, somewhat distorted, and always more or less chlorotic (Fig. 3, D). Nearly complete chlorosis of the most recently formed foliage usually was observed at this stage of disease development. Foliage produced by further growth bore the marble type of mottle pattern, which consisted of rather large chlorotic areas bounded by light green or normal green tissue. Much of the chlorotic tissue was almost colorless, as shown in figure 3, D.

Other disease symptoms observed were leaf drop on the lower part of infected plants and yellowish brown to brown stem discoloration at the nodes. Such stem discoloration appeared first at the point of leaf petiole attachment. Later, the discolored area usually completely circled the stem and then spread along the internode. Almost all varieties of peas inoculated with *pea virus 2A* developed discoloration at the nodes, but occurrence of stem discoloration along the internodes was variable.

Infected plants of Yellow Admiral and Bruce varieties did not exhibit typical symptoms. No large areas of chlorotic tissue were formed in infected foliage of Yellow Admiral. Very little if any mottle was exhibited by infected Bruce seedlings, but general yellowing of the top foliage was conspicuous. Stem discoloration was emphasized in Bruce plants. Very often this symptom appeared before any foliage symptoms were discernible.

#### Speckle Pea Mosaic (*pea virus 2B*)

Disease symptoms produced by *pea virus 2B* differed from those caused by *pea virus 2A* in type of mottle and degree of expression of the other symptoms noted. The general course of disease development was the same in both cases. The mottle pattern produced by *pea virus 2B* consisted of small irregular-shape spots of dark green tissue bounded by extensive yellowish green areas (Fig. 3, E). A few small chlorotic areas were almost colorless. Very little distortion or reduction in size of leaflets and stipules resulted, and the small amount of cortical discoloration usually was limited to the node areas. Occasional leaf drop was observed.

#### Mild Pea Mosaic (*pea virus 2C*)

The expression of disease symptoms produced by *pea virus 2C* began on the newly formed foliage as a very slight vein clearing about 8 days after inoculation of seedling plants. The next two or three sets of leaflets and stipules produced exhibited a very mild mottle (Fig. 3, F). None of the foliage ever became chlorotic enough to make the mottle pattern conspicuous. Growth of plants infected with this virus was not retarded, and no distortion of foliage occurred. Only occasionally were leaf drop and stem discoloration noted, and then only to a very limited extent. Vein clearing and slight discoloration at nodes were the only symptoms observed on Bruce plants.

#### Tobacco Ring Spot

Symptoms produced by the *tobacco ring-spot virus* on pea seedlings began with top necrosis and an occasional ring pattern on leaflets not killed (Fig. 3, C). No mottle pattern was produced like any of those resulting from infection with the pea-mosaic viruses. The upper leaflets frequently presented a netted, bleached appearance for a few days and then collapsed completely. A conspicuous brownish discoloration usually developed rapidly on the entire stem. A large percentage of infected plants died within 2 weeks after inoculation. No primary local lesions were observed on the inoculated foliage.

#### Relation of Temperature to Symptom Expression

Yellow Admiral, Mammoth Melting Sugar and White Marrowfat seedlings were inoculated with *pea viruses 2A, 2B, and 2C*. All plants were

kept at 20–22° C. until typical mosaic symptoms for each of the viruses developed on those plants successfully infected. The plants were then divided into 4 groups, each group including 4 healthy plants of each variety, and 4 infected plants of each variety for each of the 3 different viruses. One group of plants was placed in each of the four greenhouses at the following temperatures: 12–14; 18–20; 22–24; 28–30 degrees C. Small tags were attached to the tops of the plants to mark the uppermost foliage developed before the plants were moved to the various temperatures.

At the end of the first week all healthy plants were growing vigorously at the 3 lower temperatures, but only a small amount of new growth had been produced by healthy plants kept at the highest temperature. Typical mottle symptoms appeared on new growth produced by infected plants grown at 18–20, 22–24, and 28–30 degrees C.

In contrast with plants kept at higher temperatures all infected plants kept at 12–14° C. grew nearly as vigorously as healthy plants of the same variety. In plants infected with virus 2A, the amount and extent of chlorosis was markedly reduced and no large completely chlorotic areas appeared in the new foliage. In plants infected with virus 2B, reduced chlorosis resulted in a mottle consisting of vein clearing and few mildly chlorotic spots. In plants infected with *pea virus* 2C, the new foliage could not be distinguished from that of healthy plants of the same variety at this temperature. Tissue inoculation to healthy plants at 20–22° C. showed that the same virus was present in the tissues expressing typical and atypical symptoms, and all 3 viruses were recovered apparently unchanged. When plants bearing atypical symptoms were moved from 12–14° back to 20–22° C., typical symptoms appeared on the new growth produced at the latter temperature.

The temperature effect on symptoms produced by *pea virus* 1 was tested in a similar manner with infected Alderman seedlings. At 28–30° C. severe top-necrosis developed during the first 10 days of exposure and all infected plants died within 3 weeks. Healthy plants remained alive, but grew very slowly. Symptoms produced at 18–20° C. and 22–24° C. were like those previously described at 20–22° C. Two of the infected plants placed at 12–14° C. resumed growth at the terminal bud, whereas the other two reacted like plants kept at 20–22° C. The first two bore foliage exhibiting chlorotic spots and enations like those ordinarily produced on secondary shoots after the terminal bud has been destroyed, and like those produced on plants infected in the latter part of their growing period. Tissue transfers from infected plants showed that *pea virus* 1 was present and apparently unchanged in tissues showing either type of symptom, but tissue from plants kept at 28–30° C. was a poor source of inoculum. The high temperature response obtained probably was due to a direct effect of too high a temperature on the plants that had been weakened by infection. Weak seedlings not infected by the virus often succumbed at temperatures of 28–30° C.



The results obtained in these temperature studies demonstrate that low temperatures (12–14° C.) tend to mask or suppress symptoms associated with *pea viruses* 2A, 2B, and 2C. Temperatures of 18–24° C. permit rapid development of the disease and full expression of symptoms. Higher temperatures apparently influence symptoms only indirectly by reducing the vigor of the host. The similarity in symptom expression of *pea viruses* 2A, 2B, and 2C, under like temperature influences, is regarded as further evidence that they are closely related viruses or strains of a single virus.

Temperature effects upon symptom expression of *pea virus* 1 were not well defined. Low temperatures lessened the severity of the disease, but not so pronouncedly as with strains of *pea virus* 2. Terminal bud distortion was less severe at low temperatures, but the occurrence of leaf enations and transparent spots on the foliage was not changed. High temperatures (28–30° C.) increase the severity of the disease probably primarily through their detrimental effect upon host development.

#### Mosaic-like Symptoms not due to Viruses

A heritable chlorophyll deficiency, which may be confused with mosaic symptoms on bean, has been considered by Burkholder and Muller (2). Parker (19) has briefly reviewed the literature on other chlorophyll deficiencies in bean, such as variegations and albinism. The literature on the genetics of peas has been reviewed by Wellensiek (28) and includes a discussion of such chlorophyll abnormalities as albinism, pale yellow, white-green, and yellow-green variegations. Most of these heritable characters need not be confused with the symptoms of virus diseases, but they must be considered when studying seed transmission of viruses.

While examining pea seedlings for evidence of seed transmission, a few questionable plants were found. Continued growth of these plants demonstrated clearly that most of them were variegated; some being white-green and others yellow-green. A very few albinos were found. However, other plants were marked with yellow spots that, at least superficially, resembled mottle patterns from virus infection. Such material never yielded a virus and continued growth of these plants indicated clearly that a virus probably was not involved.

Other abnormalities occurred that, under some circumstances, might be confused with mosaic symptoms. Many Alaska seedlings found in seed-transmission trials had abnormal foliage. The leaflets and stipules of such plants were small, rough, sometimes crinkled, and always more or less striped or spotted with chlorotic tissue underlain by green tissue. Repeated attempts to transmit an infective agent from this type of tissue always failed.

Continued growth of these abnormal seedlings usually depended upon the development of an axillary bud because the terminal buds of such plants



appeared injured or completely destroyed. The secondary stem produced from axillary-bud development always bore normal foliage, which fact in itself materially reduced the possibility of this being a virus trouble.

Nearly all varieties of peas observed produced a few plants bearing abnormal foliage and injured terminal buds, but the condition occurred most frequently when old Alaska seed was planted in soil that tended to become compact before the seedlings emerged.

#### INSECT TRANSMISSION

When transmission studies were undertaken, the occurrence and duration of an incubation period of any pea virus in the insect vector was not known. However, incubation periods of the duration reported by Osborn (18) were supplied for all of the viruses used in these studies. Infected peas were used as a source of inoculum for the experiments reported, but the pea aphid also became infective after feeding on broad-bean or crimson-clover plants infected with the pea-mosaic viruses. The combined results of duplicated tests are recorded in table 1. Five plants served as a test unit in each case.

TABLE 1.—Transmission of pea-mosaic viruses and tobacco ring-spot virus by the pea aphid

Transferred to	Aphids from plants infected with:					Control healthy plants
	<i>Pea virus 1</i>	<i>Pea virus 2A</i>	<i>Pea virus 2B</i>	<i>Pea virus 2C</i>	<i>Ring-spot virus</i>	
<i>Pisum sativum</i> L. var.						
Perfection .....	10/9 <sup>a</sup>	10/0	10/0	10/0	10/0	10/0
var. Yellow Admiral .....	10/5	10/8	10/8	10/7	10/0	10/0
var. White Marrowfat .....	10/4	10/7	10/8	10/6	10/0	10/0
var. Mammoth Melting						
Sugar .....	10/6	10/9	10/9	10/8	10/0	10/0
<i>Vicia faba</i> L. var. <i>Minor</i>	10/5	10/5	10/5	10/3	10/1 <sup>b</sup>	10/0

<sup>a</sup> First figure is number of plants inoculated; second figure is number of plants infected.

<sup>b</sup> Virus not recovered from plant.

The results recorded in table 1 demonstrate that the pea-mosaic viruses were readily transmitted by the pea aphid. No experiments were conducted to demonstrate the length of time that aphids remain infective. However, observation during other inoculation work in which aphids were used indicated that, when infected with *pea virus 1*, they retained the virus and ability to transmit it throughout most of their lives. Other groups of aphids carrying *pea viruses 2A, 2B, and 2C* infected high percentages of pea seedlings

when transferred from diseased to healthy plants, but subsequent transfers to additional healthy plants resulted in very little infection.

No insect vector has been found for the *tobacco ring-spot virus*.

#### VARIETAL SUSCEPTIBILITY

As early as 1923, Martin and Haensler<sup>9</sup> observed an "abnormal condition similar to a mosaic", which occurred with greater severity on some varieties of peas than it did on other varieties. Little Marvel and Horsford varieties were very severely attacked, while Alaska, Thomas Laxton, and others had much less of the disease. Other observations recorded in plant-disease reports<sup>10, 11, 12, 13, 14</sup> also indicate differences in amount and severity of mosaic on peas, a part of which may have been due to varietal differences. Doolittle and Jones (7), using artificial methods of inoculation, obtained about 70 per cent infection on Telephone peas and about 40 per cent on Alaska peas. Johnson and Jones (10) report that preliminary field tests show that some varieties possess a degree of resistance. A 1932 planting of peas in the legume nursery at Madison, Wisconsin, also gave evidence of differences in amount and severity of virus infection on different varieties.

#### Field Tests

Field records on mosaic susceptibility taken from varietal plantings made in the legume nursery at Madison, in 1933, are recorded in table 2. Yellow Admiral was used as a check variety throughout the plot, because it had previously been observed to be very susceptible to some of the viroses attacking peas grown on that plot. The number and identity of the viruses infecting the peas was not known.

Very few aphids, *Macrosiphum pisi* Kalt., were present among the peas at any time during the season, although careful search did reveal an occasional aphid as early as May 25.

Assuming that aphids are the principal vectors, and knowing that they were few in number, it would be reasonable to suppose that the results obtained only grossly represent the true susceptibility to the viruses present. A planting, made later in the summer, however, probably came closer to

<sup>9</sup> Martin, W. H., and C. M. Haenseler. Pea diseases in New Jersey. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Repr. 8: 50. 1924. [Mimeographed.]

<sup>10</sup> Haskell, R. J. Diseases of field and vegetable crops in the United States in 1924. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Repr., Sup. 41. 1925. [Mimeographed.]

<sup>11</sup> Wood, J. I., N. E. Stevens and P. R. Miller. Diseases of plants in the United States in 1932. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Repr., Sup. 85. 1933. [Mimeographed.]

<sup>12</sup> Young, P. A. and H. E. Morris. Plant diseases in Montana in 1928. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Repr., Sup. 69. 1929. [Mimeographed.]

<sup>13</sup> See footnote 3, p. 242.

<sup>14</sup> See footnote 4, p. 242.

TABLE 2.—*Occurrence of mosaic resulting from natural infection in early spring planting in the field at Madison, Wisconsin, 1933*

Variety	Plants	Mosaic	Variety	Plants	Mosaic
	<i>number</i>	<i>per cent</i>		<i>number</i>	<i>per cent</i>
Alaska <sup>a</sup> .....	94	4.3	Dwarf Telephone .....	127	37.0
Yellow Admiral .....	304	19.4	Giant Wonder .....	99	0
Green Admiral .....	86	30.2	Perfection .....	207	1.0
Prince of Wales .....	113	15.0	Abundance .....	137	0
Hundredfold .....	100	0	Stratagem .....	86	13.9
Improved Gradus .....	87	11.5	Thomas Laxton .....	99	2.0
Bliss Everbearing .....	62	11.2	Alderman .....	58	29.3
Surprise <sup>a</sup> .....	92	0	Telephone .....	61	16.5
Premium Gem <sup>a</sup> .....	93	0.9	Senator .....	54	0
Nott's Excelsior .....	125	0	Canada Field Pea .....	170	9.4
Laxton's Progress .....	75	12.0	White Marrowfat .....	140	15.0
Horsford .....	115	0.9	Blackeyed Marrowfat .....	118	26.2
Ashford .....	94	1.1	Mammoth Melting .....		
Bruce .....	71	15.5	Sugar .....	88	38.6

<sup>a</sup> These varieties were nearly mature before mosaic infection became uniformly distributed over the plot.

indicating the total percentage of susceptible plants for the varieties tested. During early September, 1933, the aphid population among these peas was sufficient to damage them slightly. The results obtained from this small planting are given in table 3.

TABLE 3.—*Occurrence of mosaic resulting from natural infection in late summer planting in the field at Madison, Wisconsin, 1933*

Variety	Plants	Mosaic
	<i>Number</i>	<i>Per cent</i>
Perfection .....	31	0
Bruce .....	33	85
Yellow Admiral .....	48	97
White Marrowfat .....	34	100
Mammoth Melting Sugar .....	32	100

The results obtained in field trials indicated that some varieties of peas were highly resistant to some or all of the viruses present. Some early-maturing varieties that showed little or no disease may have been resistant or they may have escaped infection. The varietal tests made in the greenhouse have shown more definitely that some varieties were resistant to some of the viruses known to be present in the field trial-plot area. Other varieties not infected in the field were susceptible, but apparently escaped infection or disease development because of their early maturity.

TABLE 4.—*Reaction of thirty-four varieties of peas to inoculation with viruses in the greenhouse<sup>a</sup>*

Variety of <i>Pisum sativum</i> L.	Pea virus 1		Pea virus 2A		Pea virus 2B		Pea virus 2C		Ring- spot virus	
	Plants inoculated (number)	Plants infected (per cent)	Plants inoculated (number)	Plants infected (per cent)	Plants inoculated (number)	Plants infected (per cent)	Plants inoculated (number)	Plants infected (per cent)	Plants inoculated (number)	Plants infected (per cent)
Perfection .....	30	43	53	0	57	0	59	0	26	61
Giant Wonder .....	17	18	35	0	35	0	35	0	14	50
Abundance .....	28	11	25	0	10	0	9	0	10	40
Ashford .....	15	13	50	0	50	0	50	0	15	53
Nott's Excelsior .....	9	33	38	0	18	0	36	0	13	62
Hundredfold .....	40	30	24	0	38	0	16	0	40	45
Alaska .....	20	45	20	20	20	5	20	10	20	40
Yellow Admiral .....	41	10	43	30	47	43	46	35	37	49
Green Admiral .....	8	38	16	31	16	6	25	24	4	100
Prince of Wales .....	10	20	20	30	25	12	20	20	20	40
Improved Gradus .....	25	40	25	36	25	24	25	40	25	56
Gradus .....	18	50	35	54	35	23	34	59	18	50
Bliss Everbearing .....	13	39	25	24	20	30	24	25	20	70
Referendum .....	16	13	35	87	35	29	35	40	8	75
Surprise .....	12	25	10	60	15	33	12	42	13	77
Premium Gem .....	12	42	13	31	13	23	15	31	15	47
Laxton's Progress .....	10	50	35	61	35	49	35	31	14	36
Horsford .....	20	80	22	5	20	25	25	28	15	33
Bruce .....	20	70	18	22	14	29	25	56	12	75
Dwarf Telephone .....	27	59	30	40	30	27	30	37	20	60
Stratagem .....	18	28	24	38	32	53	19	58	13	77
Stratah .....	32	22	29	59	30	33	30	30	35	67
Stridah .....	26	19	24	33	25	24	24	21	17	77
Icer .....	6	50	26	35	20	40	20	5	3	100
Champion of England .....	10	20	20	45	24	21	22	23	13	62
Thomas Laxton .....	10	10	30	40	30	27	30	60	10	30
Alderman .....	47	40	48	60	42	45	41	40	20	50
Senator .....	9	33	20	15	20	10	25	28	7	29
Canada Field Pea .....	34	41	45	82	45	62	45	36	35	41
Blackeyed Marrowfat .....	10	40	25	12	15	33	14	29	14	36
White Marrowfat .....	16	25	24	33	15	7	25	36	20	80
Dwarf Gray Sugar .....	30	20	20	30	20	60	20	50	27	19
Mammoth Luscious Sugar .....	20	65	50	60	34	40	32	41	18	61
Mammoth Melting Sugar .....	25	16	48	48	26	27	22	23	26	54

<sup>a</sup> Each plant inoculated once by the tissue-insertion method.

## Greenhouse Tests

Early in these studies Perfection and Yellow Admiral seedlings were used in experiments on methods of mechanical transmission of *pea viruses* 2A, 2B, and 2C. When the tissue-insertion method of inoculation proved moderately successful on Yellow Admiral, but failed completely on Perfection, it seemed

advisable to obtain information concerning the reaction of other varieties of peas to these viruses. Furthermore, these results were in accord with the indications of varietal differences found in field trials. Thirty-four varieties and strains of peas were inoculated with *pea viruses* 2A, 2B, and 2C by the tissue-insertion method. Later, the same varieties were inoculated with *pea virus* 1 and the *tobacco ring-spot virus* by the same method. The total number of plants of each variety inoculated with the different viruses and the percentage infection obtained in each case are recorded in table 4.

Examination of the data presented reveals that *pea viruses* 2A, 2B, and 2C failed to infect Hundredfold, Nott's Excelsior, Giant Wonder, Perfection, Abundance, and Ashford seedlings. All other varieties inoculated with these 3 viruses were infected in varying percentages. All varieties tested were found to be susceptible to *pea virus* 1 and the *tobacco ring-spot virus*.

Wide variations in the percentage of infection obtained when studying the same virus on different varieties of the host may have some significance. However, the author has chosen to place emphasis on those cases only in which no infection was obtained. Since all plants were not grown at the same time and the same infected material could not be used for inoculum each time, it was impossible to determine how much of the variation in percentage of infection was due to the host alone. Furthermore, it was later found that where the tissue-insertion method of inoculation produced only 40 to 60 per cent infection on certain varieties, the carborundum method of inoculation produced nearly 100 per cent infection with the same virus on the same variety. The writer does not mean to imply that all varieties infected with the different viruses are 100 per cent susceptible to those viruses. Only in those cases where no infection was obtained are the percentages of infection regarded as fairly accurate indices of resistance.

Attempts were made to infect the 6 resistant varieties of peas with *pea viruses* 2A, 2B and 2C by the carborundum method of inoculation. No infections were obtained. Attempts to infect Perfection seedlings with these viruses also failed when infective aphids were used as inoculation agents. *Pea viruses* 2A, 2B and 2C have never been recovered from inoculated seedlings of the 6 varieties regarded as resistant.

#### HOST RANGE

The pea aphid was used as a means of inoculating the clovers and alfalfa with the pea-mosaic viruses, and the carborundum method was used to inoculate the clovers and alfalfa with the *tobacco ring-spot virus*. Other hosts studied were inoculated with all of the viruses studied by the carborundum method. Table 5 embodies a summary of the hosts infected with the different viruses studied. The virus was recovered from all hosts recorded as susceptible.

TABLE 5.—Results of host range studies with pea-mosaic viruses and tobacco ring-spot virus

Species inoculated	Pea virus 1	Pea virus 2A	Pea virus 2B	Pea virus 2C	Ring- spot virus
<i>Phaseolus vulgaris</i> L. var. Refugee Green	—	—	—	—	+
var. Corbett Refugee .....	—	—	—	—	+
var. Red Valentine .....	—	—	—	—	+
<i>Vigna sinensis</i> Endl. var. Iron .....	—	—	—	—	—
<i>Soja max</i> Piper var. Midwest .....	+	—	—	—	+
<i>Vicia faba</i> L. var. minor .....	+	+	+	+	+
<i>Lupinus alba</i> L. ....	—	+	+	+	+
<i>Lathyrus odoratus</i> L. ....	+	+	+	+	+
<i>Nicotiana tabacum</i> L. var. Havana #38 .....	—	—	—	—	+
<i>N. glutinosa</i> L. ....	—	—	—	—	—
<i>Lycopersicon esculentum</i> Mill. var. Globe	—	—	—	—	—
<i>Trifolium pratense</i> L. ....	—	—	—	—	—
<i>T. hybridum</i> L. ....	—	—	—	—	—
<i>T. repens</i> L. ....	—	—	—	—	—
<i>Medicago sativa</i> L. var. Common .....	—	—	—	—	—
<i>Melilotus officinalis</i> Willd. ....	+	+	+	+	+
<i>M. alba</i> Desr. ....	—	—	—	—	+
<i>T. incarnatum</i> L. ....	+	+	+	+	+

No differences in host range or reaction on the susceptible hosts were found between *pea viruses* 2A, 2B, and 2C. Broad bean, white lupine, *Lupinus alba* L., sweet pea, yellow sweet clover, and crimson clover were all infected systemically with these 3 viruses. No primary or secondary local necrotic lesions were observed. Mottling of the new foliage was the only disease symptom produced.

*Pea virus 1* infected all but white lupine of those hosts found for the other pea viruses studied, and in addition it infected soybean, *Soja max* Piper. Systemic infection with mottling or spotting was observed on all of the hosts infected. Crimson clover expressed a symptom very much like the spotting produced on pea foliage when *pea virus 1*-infected seedlings developed secondary shoots or continued to grow from the terminal bud. Enations occurred around the spots and along the veins on the lower surface of the leaves. This host range coincides with that of the virus used by Osborn (18) the symptoms of which were similar to those described here as enation mosaic.

Of the hosts found for the *tobacco ring-spot virus* only pea and sweet pea were not previously reported. Application of tobacco ring-spot inoculum by tissue insertion or expressed juice plus carborundum gave abundant infection during these studies.

Symptoms observed on sweet pea were like those previously described for the tobacco ring spot on peas. The symptoms observed on other hosts were the same as previously reported by Pierce (20) and Wingard (30).

#### PHYSICAL PROPERTIES OF THE VIRUSES

Expressed juice from recently infected pea seedlings was used in making property studies of the pea-mosaic viruses. Dilutions were made with sterile distilled water and the inoculum applied immediately with a cheesecloth pad, using carborundum as an abrasive. Expressed juice for aging studies was stored in stoppered test tubes at 20–22° C. and the inoculum applied in the same manner. Twenty Alderman seedlings served as a test unit in each case. The data presented in tables 6 and 7 are the total of a number of tests made at different times with different virus samples.

TABLE 6.—*Effect of dilution on the infectivity of extracted juice from pea plants affected with pea-mosaic viruses*

Virus	Dilution							
	0	$\frac{1}{250}$	$\frac{1}{500}$	$\frac{1}{750}$	$\frac{1}{1000}$	$\frac{1}{1500}$	$\frac{1}{2000}$	$\frac{1}{3000}$
<i>Pea virus 1</i> .....	60/36 <sup>a</sup>	20/13	20/13	40/16	60/13	60/8	60/2	60/0
<i>Pea virus 2A</i> .....	60/44	20/9	20/8	40/6	40/6	60/1	60/0	60/0
<i>Pea virus 2B</i> .....	60/42	20/10	20/6	40/4	60/4	60/0	60/0	.....
<i>Pea virus 2C</i> .....	60/30	20/5	20/1	40/1	60/3	60/0	60/0	.....

<sup>a</sup> First figure is number of plants inoculated; second figure is number of plants infected.

TABLE 7.—*Effect of aging in vitro on the infectivity of extracted juice from pea plants affected with pea-mosaic viruses*

Virus	Interval of aging					
	0 hours	12 hours	24 hours	48 hours	72 hours	96 hours
<i>Pea virus 1</i> .....	40/36 <sup>a</sup>	20/19	20/19	20/16	40/14	40/0
<i>Pea virus 2A</i> .....	40/35	40/3	40/0	.....	.....	.....
<i>Pea virus 2B</i> .....	40/22	40/6	40/0	.....	.....	.....
<i>Pea virus 2C</i> .....	40/10	40/1	40/0	.....	.....	.....

<sup>a</sup> First figure is number of plants inoculated; second figure is number of plants infected.

Data in tables 6 and 7 show that *pea virus 1* possesses physical properties quite different from those of the other viruses studied, and that the properties studied failed to reveal any significant differences between *pea viruses 2A*, *2B* and *2C*. Under the conditions studied, *pea virus 1* was slightly infective at 1–2000 dilution, but inactivated by 1–3000 dilution. It gave 35 per cent

infection after 3 days aging *in vitro*, but was inactivated by the fourth day. *Pea viruses 2A, 2B and 2C* tolerated 1-1000 dilution. Only *2A* produced infection at 1-1500 dilution and then only 1 plant out of 60 was infected. These 3 viruses reacted alike to aging, each being mildly infective after 12 hours aging, but inactivated by 24 hours aging.

The properties of the *tobacco ring-spot virus* as determined by several workers (8, 11, 20, 22), are distinctly different from those of the pea mosaic virus as described above. Inactivation by dilution occurs at 1-1000 to 1-10,000, while inactivation *in vitro* occurs in 5 to 14 days.

#### SEED TRANSMISSION

Stewart and Reddick (25) were the first to demonstrate that common bean mosaic is transmitted through the seed of *Phaseolus vulgaris* L. their findings having since been substantiated by others. Many other legumes have been reported to transmit virus diseases through their seed, but few of these reports have been confirmed. The increasing importance of pea mosaic in certain localities and the conflicting evidence recorded, prompted an investigation of the possible seed-borne nature of pea mosaic.

Twenty-seven varieties of peas were planted in a legume nursery plot at Madison, Wisconsin, in 1933. Mosaic plants were tagged for identification at maturity. Symptom differences were noted among the infected plants, but variations within (and between) varieties, combined with early and late infections, made classification or grouping unreliable. Consequently, all types of mosaic symptoms were treated alike when seed was gathered for seed-transmission experiments. The following year, 36 varieties were planted on the same plot, but only a few became diseased early enough to warrant gathering their seed for further trials. Observation of symptoms and transmission to greenhouse plants demonstrated that the 4 pea-mosaic viruses described earlier in the paper were present in the plot from which these seed samples were collected. The proportion of plants infected with each of the different viruses was not determined.

Greenhouse plantings of the seed collected in the summer of each year were begun the following September and continued through the winter until early spring. Some of the material was grown in pots or flats under cages, but most of it was planted in uncovered bench space. The seedlings usually appeared above ground in 6 to 8 days and were ready for close examination in 12 to 14 days. Final observation of the plants was made about 4 weeks after planting. All doubtful plants were tagged and allowed to grow for further observation. From the 1933 seed 4011 seedlings were grown, and from the 1934 seed, there were 3060 seedlings. Only 3 virus-infected seedlings were found.



Three questionable plants of Mammoth Melting Sugar variety, which occurred in one of the many plantings made, were tagged as doubtful when first observed, but later they developed unmistakable mosaic symptoms. No aphids were observed on any of these plants or any others about them. All that can be said is that these plants were not caged, and it would be possible for aphids to infect them and for symptoms to develop soon after the first observation. Each of the 3 seedlings showing mosaic symptoms belonged to a different single plant progeny and each exhibited a distinct type of mosaic symptom. The 3 diseased individuals were saved for studies discussed earlier in this paper.

Seed from mosaic-infected peas was received from W. C. Snyder in California, and Irving J. Courtice of the State of Washington. Mosaic-infected Canner's Gem peas at Madison, Wisconsin, also yielded seed for seed-transmission trials. Two samples of Alderman seed from Washington were seed-stock remnants. These stocks were suspected of having transmitted mosaic through the seed. A total of 2637 seedlings was produced from 1933-grown seed and 3620 were obtained from 1934-grown seed, but careful examination failed to reveal any mosaic-infected seedlings.

The identity of the virus or viruses infecting the plants that produced the seed for the above trials is not known to the author. However, observation of specimens and successful transfers from fresh specimens sent from California and Washington suggest that the *pea virus 1* already described occurs on peas in those States. Snyder's (24) description of mosaic-infected peas in California also indicates the presence of this virus in that State.

Seeds tested for transmission of pea mosaic came from naturally infected plants grown in Wisconsin, Washington, and California. Twenty-two varieties of peas were represented. The occurrence of only 3 questionable cases of seedling infection from seed out of 13,328 individuals examined is evidence that seed-transmission of pea-mosaic diseases is rare, if it occurs at all. Johnson and Jones (10) came to the same conclusion.

SUMMARIZED DESCRIPTIONS OF PEA-MOSAIC VIRUSES STUDIED  
Enation Pea Mosaic (*pea virus 1*)

*Only Leguminous Hosts Known.* Infects peas, including Perfection variety, crimson clover, broad bean, soybean, sweet pea, and yellow sweet clover, but not red clover, garden bean, or white lupine. Transmitted by pea aphid, *Macrosiphum pisi* Kalt. Not readily transmitted by plant extract (except with an abrasive). Transmitted by tissue insertion in stem. Inactivated by 4 days' aging at 20–22° C. Inactivated by 1 to 3000 dilution. Distribution: United States. Produces mottling or spotting of foliage on peas, dwarfing, distortion of pods and foliage, foliage enations.

### Marble Pea Mosaic (*pea virus 2A*)

*Only Leguminous Hosts Known.* Infects some varieties of peas (not including Perfection variety), crimson clover, broad bean, sweet pea, yellow sweet clover, and white lupine, but not red clover, garden bean or soybean. Transmitted by pea aphid, *Macrosiphum pisi* Kalt. Not transmitted by plant extract (except with an abrasive). Transmitted by tissue insertion in stem. Inactivated by 1 day's aging *in vitro* at 20–22° C. Inactivated by about 1–1500 dilution. Distribution: United States and probably Europe. Produces mottling with chlorosis of pea foliage, dwarfing, leaf drop, stem discoloration at nodes, but neither pod distortion nor foliage enations.

### Speckle Pea Mosaic (*pea virus 2B*)

Same host range, pea varietal reaction, mode of insect and mechanical transmission, and physical properties as *pea virus 2A*. Distinguished by speckled mottle with less chlorosis, and other symptoms more mild than *pea virus 2A*.

### Mild Pea Mosaic (*pea virus 2C*)

Same host range, pea varietal reaction, mode of insect and mechanical transmission, and physical properties as *pea virus 2A*. Distinguished by very mild mottle, very little dwarfing, leaf drop, or stem discoloration at nodes.

### DISCUSSION

Pierce (20) has shown that under field conditions beans are infected by two distinctly different viruses. He has described the two diseases and made studies that make it possible to distinguish *bean virus 1* and *bean virus 2*, causing "common bean mosaic" and "yellow bean mosaic", respectively, on the basis of host reactions and physical properties of the viruses. It has been demonstrated experimentally that the *tobacco ring-spot virus*, *alfalfa virus 2*, the *sugar beet curly-top virus*, and other viruses infect bean, but of these viruses only the *sugar beet curly-top virus* has been shown to infect beans under field conditions.

Of the above mentioned viruses, only *bean virus 1* and the *sugar beet curly-top virus* have not been demonstrated to be infectious to peas. Osborn (18), Johnson and Jones (10), Zaumeyer and Wade (32, 33, 35) and Pierce (20, 21) have all recognized two or more distinct viruses from pea. As previously stated, Osborn's pea-mosaic virus, which produced enations, is believed to be identical with the virus here designated as *pea virus 1*. The "common mosaic" and "severe mosaic" of peas reported by Johnson and Jones (10) are not yet sufficiently characterized to permit concluding that they are identical with or different from any of the well-described legume

viruses or the pea-mosaic viruses described in this paper. Pierce (21) established that *bean virus 2* infects pea. This discovery undoubtedly explains, in part at least, why so many workers have reported the occurrence of a pea-mosaic virus that infected beans, red clover, and other leguminous hosts. Pierce's description of *pea virus 3*, which causes "Common Pea Mosaic", establishes the identity of another pea-mosaic virus that infects most varieties of peas, broad bean, yellow sweet clover, and red clover, but not beans. It may be that this is the same virus reported by Dickson (5), Doolittle and Jones (7), Böning (1), Chamberlain (3), and Merkel (15). It also is very probable that the viruses designated by Zaumeyer and Wade (33) as "pea-mosaic virus 1" and "red-clover-mosaic virus" are the same as *pea virus 3* described by Pierce (21). Data presented by Zaumeyer and Wade (33) on pea-mosaic virus 2, white sweet clover mosaic, and yellow sweet clover mosaic, suggest that these three mosaic diseases were caused by the yellow bean mosaic virus (*bean virus 2*). Their alsike-clover mosaic, and sweet-pea mosaic may or may not have been caused by *bean virus 2*.

A virus taken from mosaic-diseased Perfection peas has been designated as *pea virus 1* and the disease caused by it named *Enation Pea Mosaic*. This virus has not infected garden beans, red clover, or solanaceous hosts during these studies. Its hosts and properties are different than those of *bean virus 1*, *bean virus 2*, and *pea virus 3* and no positive evidence has been obtained that this virus is seed-borne in peas. Even though it does infect soybean, it probably is not identical with the virus causing soybean mosaic described by Kendrick and Gardner (13), since their virus did not infect field peas.

*Pea viruses 2A, 2B, and 2C* also differ significantly in hosts and properties from *bean virus 1*, *bean virus 2*, and *pea virus 3* and do not infect the solanaceous hosts tested. These three viruses have the same hosts and properties, so far as studied. Their possible seed-borne nature has not been proved or disproved, but the present evidence indicates very little or no transmission with the seed. All three of these viruses failed to infect Perfection, Hundredfold, Nott's Excelsior, Ashford, Giant Wonder, and Abundance varieties of peas. *Pea virus 1* infects these and all other pea varieties tested. Other host differences noted were that *pea viruses 2A, and 2B, and 2C* infected white lupine, but *pea virus 1* did not, and the latter infected soybean, but the *pea virus 2* group did not. Similarly, *pea viruses 2A, 2B, and 2C* differed significantly from *pea virus 1* in properties studies.

Since the three viruses of the *pea virus 2* group were found to be alike in host range, reaction on pea varieties, physical properties, and modes of transmission, they probably are best regarded as strains of the same virus until significant differences are found to characterize each. Other hosts known to be infected with two or more distinct viruses, one of which is represented by symptomatologically different strains, are tobacco, cucumber, and potato.

Still other viruses cause a streak type of disease symptom, as reported by Linford (14) and Whipple (29). The viruses that Zaumeyer and Wade (32) transmitted from white sweet clover and white clover to peas caused streak-like symptoms, but the viruses involved have not been associated with any well-described virus known to attack legumes. Pierce (20) found that *alfalfa virus 2* infects peas, but the symptoms were not recorded. The writer, however, has inoculated peas with *alfalfa virus 2* and observed symptoms similar to those reported recently by Zaumeyer and Wade (34) when an alfalfa virus infected peas.

Field and greenhouse studies on susceptibility of 34 varieties of peas to *pea virus 1* and the 3 strains of *pea virus 2* show that many canning and market-garden varieties are susceptible to these mosaic diseases, but, under Wisconsin conditions, some escape severe injury. Many such varieties are well-developed or mature before mosaic infection is spread by the pea aphid. These same varieties, however, planted late in regions having long growing seasons, would be subject to considerable injury from mosaic.

These studies and the observations of Jones and Linford (12) and Zaumeyer and Wade (33) indicate that migration of aphids from biennial or perennial legume hosts of the pea-mosaic viruses is the important source of early spring infection. Subsequent spread of the viruses is brought about principally by aphid migrations from peas to peas. Southern or coastal regions with long growing seasons are particularly favorable for the development of pea-mosaic diseases, because spring and fall crops of peas or long-season varieties are used.

Reduction of aphid infestation, and eradication or avoidance of other susceptible legume hosts are preventive measures to be considered at all times.

#### SUMMARY

The studies in this paper were concerned primarily with the identification and description of certain viruses attacking *Pisum sativum*. Symptomatically different pea-mosaic viruses and the *tobacco ring-spot virus* were studied. One pea-mosaic virus was found to be sufficiently different from other legume-mosaic viruses to justify designating it as a distinct virus (*pea virus 1*). Three other pea-mosaic viruses were found to differ from one another in symptoms on peas, but were alike in all other characteristics studied. It is believed that the evidence obtained was sufficient to justify the conclusion that those were strains of the same virus. They were designated as *pea virus 2A*, *pea virus 2B*, and *pea virus 2C*.

Symptoms of enation pea mosaic (*pea virus 1*), marble pea mosaic (*pea virus 2A*), speckle pea mosaic (*pea virus 2B*), mild pea mosaic (*pea virus 2C*), and *tobacco ring-spot virus* infection on Alderman pea seedlings were described. All viruses studied produce systemic infection on peas.

The pea-mosaic viruses studied were readily transmitted by the pea aphid, *Macrosiphum pisi* Kalt., and by plant extract when carborundum was used as an abrasive.

The 34 varieties of peas inoculated were all more or less susceptible to *pea virus 1* and the *tobacco ring-spot virus*. *Pea viruses 2A, 2B and 2C* infected all but 6 varieties of peas inoculated.

Studies of physical properties of the pea-mosaic viruses showed that *pea virus 1* was inactivated by 1 to 3000 dilution and 4 days' aging *in vitro*, while the 3 strains of *pea virus 2* were inactivated by 1 to 1500 dilution and 24 hours' aging *in vitro*.

In seed transmission tests involving 13,328 seedlings, only 3 questionable cases of seedling infection from seed were observed.

Host range studies showed important differences between *pea virus 1* and the 3 strains of *pea virus 2*. Peas and sweet peas were the only hosts not previously reported for the *tobacco ring-spot virus*.

Since viruses other than those described in this paper are believed to cause pea-mosaic diseases, it is hoped that the data presented in this paper will be of value in establishing the number and identity of the viruses that attack peas and other legumes.

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# QUANTITATIVE STUDIES OF TOBACCO-MOSAIC VIRUS INACTIVATION BY ULTRA-VIOLET LIGHT<sup>1</sup>

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## INTRODUCTION

Radiant energy has been shown to cause mutations in several different species of animals and plants. Nearly the whole spectrum has an effect, but some wave lengths, especially the shorter ones, are more effective than others. Since viruses are known to mutate (8, 9, 16) and since they also resemble the genes of higher animals and plants in some other respects, it seemed possible that radiant energy might cause mutations in this class of materials. The writers have experiments in progress designed to test this possibility. The effectiveness of ultra-violet light and X-rays in producing gene mutations is dependent upon subjection of the genes to sublethal dosages of these waves. As a preliminary to an investigation of the production of virus mutations, it was, therefore, considered necessary to determine the effect of radiant energy on inactivation of the virus. A study has been made of the effect of ultra-violet light waves on inactivation of tobacco-mosaic virus either wet or dried, purified, or in the natural plant juice. The data that follow deal with this problem.

## REVIEW OF LITERATURE

The interpretation of the cause of death of an organism irradiated by X-ray, radium, or ultra-violet light, as being due to absorption of energy in discrete units within a relatively small vital spot, has been suggested independently by several investigators (5). Absorption of energy in this manner predicates certain results. If one unit is sufficient to inactivate, then the logarithms of the survival ratios have, in terms of the probability theory, a linear relation to the total energy absorbed. If, on the other hand, more than one unit acting on the vital spot is necessary for inactivation, the logarithms of the survival values show a curvilinear relation to the energy dosage.

Curves of the single absorption type for inactivation have been observed by Wyckoff for the colon bacillus, *Bacillus coli*, and for the mouse typhoid organism, *B. aertrycke* (21, 22, 23, 24). These curves have a characteristic pattern, whether the radiation is in the cathode ray, X-ray, or ultra-violet

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band. Curves of the same form have been obtained by Gowen and Gay (6), Oliver (14), and others (5) for the rate at which genes are changed when subjected to X-rays. The case of the genes is of particular significance, since in this instance it is probable that only one characteristic unit of the cell, having a maximum size of the order of a large protein molecule (6), is the test subject.

Curves of the multiple absorption type for inactivation are observed in bacterial colonies containing more than one organism (21, 22, 23, 24), in staphylococci that stick together in chains, and in multicellular organisms, such as seeds of higher plants or animal eggs (5).

Technical difficulties enter into the demonstration of these different curve types. For example, to mention only two, the organisms must be separate from one another, and, secondly, the length of exposure to the radiation must be shorter than the time required for reproduction of the organisms. Tobacco-mosaic virus offers favorable material for study, since it apparently is composed of single infectious units (10), since these units or particles do not seem to clump together in dilute solution, and since the virus does not reproduce outside of the host plant. The type of survival curve obtained when a virus is exposed to radiation is of significance in understanding the nature of its ultimate particles.

Since Finsen and Dreyer (3) first demonstrated the virucidal action of ultra-violet light, numerous studies have been made on the relative resistance of various bacteria, bacteriophages, and viruses to ultra-violet irradiation. The results reported by the different workers are not entirely in agreement; some state that bacteriophages and viruses are less readily inactivated than bacteria (2); others that they are more readily inactivated (11); and still others that the energy required for inactivation is approximately the same for bacteriophages, viruses, and bacteria (13, 17). Apparently, viruses and bacteriophages vary in susceptibility to radiant energy, some being more readily inactivated than others. In general, it has been found that wave lengths of 2650 Å or less are more efficient than longer wave lengths (13, 17, 2). The rate of inactivation appears to depend upon the dilution of the virus or bacteriophage (4), and upon the density of the medium in which it is suspended (11).

Several investigators have shown that ultra-violet light is lethal for tobacco-mosaic virus. The time required for inactivation has been variously estimated at from 15 seconds (1) to 1 or more hours (12, 18, 7). It appears to depend on the freedom of the virus suspension from extraneous material, as well as on the intensity of the irradiation. Hollaender and Duggar (7) have reported that the most efficient part of the ultra-violet spectrum for inactivation of tobacco-mosaic virus is 2250 Å.



## MATERIALS AND METHODS

The tobacco-mosaic virus used was a single-lesion strain isolated by Jensen (8) by successive passages through necrotic primary lesions in *Nicotiana glutinosa* L. It was transferred to young Turkish tobacco (*N. tabacum* L.) plants which were allowed to become thoroughly mottled. Diseased plants were ground in a food chopper and the juice was extracted from the pulp by passage through a single layer of cheesecloth. The juice was adjusted to pH 7 by addition of approximately 3 grams of disodium phosphate per 100 cc., then filtered through a layer of celite (Hyflo Standard-cel) on a Whatman No. 42 filter paper, and subsequently passed through a Berkefeld filter candle of the "N" grade. The material was kept frozen until used for experimentation. Healthy juice was similarly prepared from healthy Turkish tobacco plants. The crystalline tobacco-mosaic virus protein used was prepared from the single-lesion strain of tobacco-mosaic virus by the method described by Stanley (19, 20). After dialysis this material was found to contain 9.65 mg. protein-nitrogen per cc. It was diluted 1:4 with 0.1 molar potassium phosphate solution at about pH 7 and kept frozen until used.

Before being exposed to ultra-violet light, the stock solutions were further diluted with 0.1 M phosphate solution at about pH 7. A mixture of crystals and healthy juice was prepared by adding 1 cc. of the stock solution of crystals and 1 cc. of the stock solution of healthy juice to 8 cc. of the 0.1 M phosphate solution, giving a final dilution for the virus of 1:10 (or 1:40 of the original preparation).

The source of ultra-violet light was a Cooper Hewitt mercury lamp. The spectrum of this lamp,<sup>2</sup> indicated in figure 1, commences at 2175 Å. It has strong lines at 2260 Å, 2285 Å, 2305 Å, 2325 Å, etc. The lamp was operated on a direct current of 110 volts. All exposures to be reported were made at a distance of 7½ inches from the horizontal bulb. At this distance some provision had to be made to eliminate the effects of heat rays. The dishes containing the virus material were placed on the top of an 8½-inch wire mesh basket and a current of air was blown across the top of and through the basket by means of an electric fan. This method of cooling was so effective that a rise of but 3 or 4 degrees over room temperature (20° C.) was obtained at the position of exposure to the light. The cooling was found to have an effect on the resistance of the lamp. Under normal running conditions, after the lamp had steadied down, the current across the lamp with the fan turned off was 84 volts. When the fan was turned on, the current across the lamp became steady at 60 volts. The ergs incident to the virus material at the position of the dish were about 10,940 per second per square millimeter of area.

<sup>2</sup> The writers are indebted to Dr. R. W. G. Wyckoff for making this spectrum.

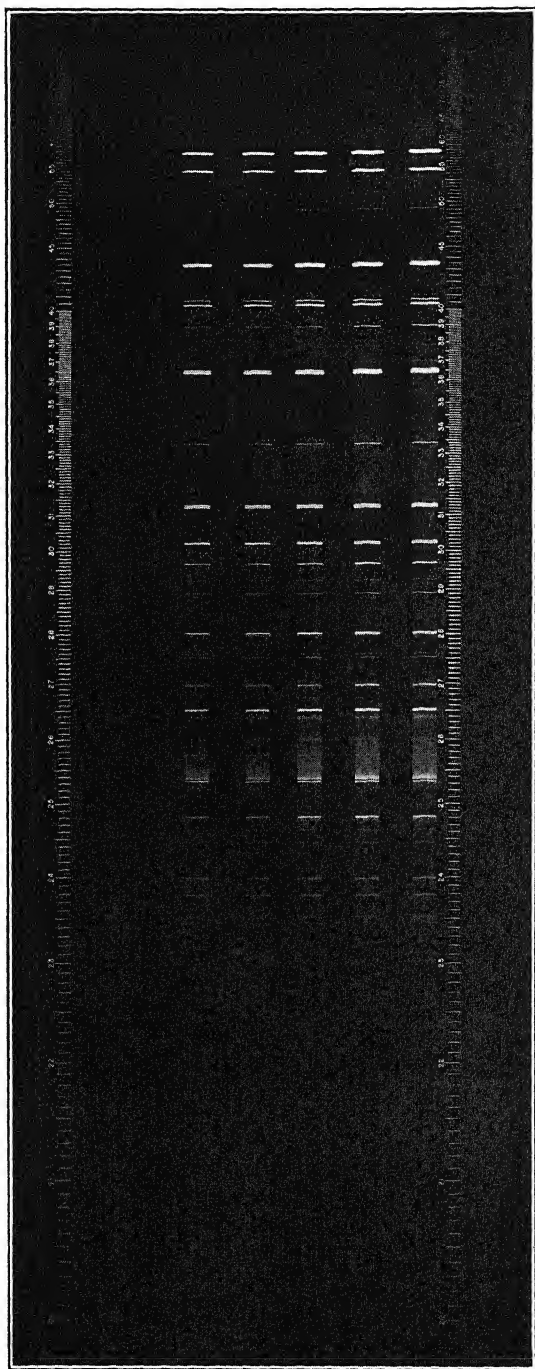


FIG. 1. Spectrum of the ultra-violet lamp used in the investigations. Figures on the scale represent the wave length in hundreds of Angstrom units.

The virus samples were exposed by placing 1 cc. of the 1:10 dilution in Syracuse watch glasses, the average diameter of which was  $49.33 \pm 1.05$  mm. After exposure, the solutions were taken up in 4 cc. of the 0.1 M phosphate solution and tested for infectivity by inoculation of 16 leaves of Early Golden Cluster bean, *Phaseolus vulgaris* L. (15). The beans were planted 2 to a pot in 4-inch pots and were used when about 10 days old, only the 2 primary leaves being inoculated. In one instance, which will be noted later, each sample was tested on 32 bean leaves.

In determining the rate of inactivation of virus exposed to ultra-violet light, 4 different types of preparations were used; juice of diseased plants, a solution of crystalline material, a solution of crystalline material to which juice of healthy plants was added, and juice of diseased plants dried down to a thin film. These 4 types were made from the stock solutions mentioned above. At least 4 independent tests were made with each type of preparation. The data obtained allow analysis of the variance due to the virucidal effect of the ultra-violet radiation as distinct from the variance within and between the different tests.

#### EXPERIMENTAL

##### Juice of Diseased Plants

The results of 4 tests performed with juice of diseased plants are given in table 1.

TABLE 1.—*Survival of tobacco-mosaic virus exposed to ultra-violet light. The virus was contained in filtered juice of diseased plants*

Time of exposure	Test 1	Test 2	Test 3	Test 4	Total
0 seconds	2475 <sup>a</sup> $\pm$ 310.4 <sup>b</sup>	1625 <sup>a</sup> $\pm$ 256.8 <sup>b</sup>	2211 <sup>c</sup> $\pm$ 211.5 <sup>d</sup>	599 <sup>a</sup> $\pm$ 87.4 <sup>b</sup>	6910
5 "	—	980 $\pm$ 124.8	1826 $\pm$ 195.2	451 $\pm$ 68.3	3257
10 "	—	532 $\pm$ 104.3	631 $\pm$ 81.0	331 $\pm$ 100.2	1494
20 "	603 $\pm$ 70.7	381 $\pm$ 43.4	595 $\pm$ 67.8	353 $\pm$ 62.9	1932
40 "	386 $\pm$ 53.8	303 $\pm$ 45.9	524 $\pm$ 75.5	71 $\pm$ 19.5	1284
60 "	59 $\pm$ 23.8	265 $\pm$ 39.4	401 $\pm$ 57.3	45 $\pm$ 10.6	770
80 "	55 $\pm$ 13.8	125 $\pm$ 15.5	407 $\pm$ 57.6	18 $\pm$ 5.9	605
100 "	32 $\pm$ 5.1	147 $\pm$ 18.2	192 $\pm$ 25.6	10 $\pm$ 3.0	381
120 "	5 $\pm$ 1.9	31 $\pm$ 6.1	79 $\pm$ 12.8	4 $\pm$ 1.8	119
140 "	2 $\pm$ 1.4	4 $\pm$ 2.4	70 $\pm$ 14.4	1 $\pm$ 1.0	77
200 "	—	2 $\pm$ 1.4	12 $\pm$ 3.5	0	14
300 "	—	2 $\pm$ 1.4	10 $\pm$ 3.2	0	12

<sup>a</sup> Numbers of lesions produced in 16 bean leaves.

<sup>b</sup> Standard error of the mean multiplied by 16, the number of leaves used for test.

<sup>c</sup> Numbers of lesions produced in 32 bean leaves.

<sup>d</sup> Standard error of the mean multiplied by 32, the number of leaves used for test.

The graphical presentation of these results, together with those obtained with the 3 other types of preparations, are given in figure 2.

The results accord with the view that ultra-violet light inactivates tobacco-mosaic virus in such a manner that the logarithm of the survival ratio is linearly decreased as the energy incident to the virus is increased. For high-speed electrons, this result suggests that a single virus entity is inactivated at every absorption of one unit of energy.

It is of interest to allocate the variances due to the various causes and see the extent to which the probability hypothesis of radiation absorption may account for the observed results. The unequal number of observations in test 1, as compared with the other tests, combined with the fact that additional numbers of leaves were used for test 3, introduces some heterogeneity into a direct analysis of the data. There are 3 possible ways in which the data may be treated, none of which gives exactly the same numerical values that would be obtained had the same number of observations been made and the same number of leaves used for each test. It is possible to analyze the entire mass of data, using averages wherever unequal numbers appear; to omit the 5-, 10-, 200-, and 300-second observations entirely, using averages again wherever unequal numbers occur; or to omit test 1 entirely and arbitrarily divide test 3 into two equal parts. All 3 types of analysis were made. While the values obtained for the 3 types were not identical, they were so nearly alike that the same conclusions are to be drawn from each. Data derived by means of the third type of analysis are given below.

	Degrees of freedom	Sum of squares	Mean square
Between ultra-violet treatments .....	11	334,776	30,434
Between tests .....	3	17,143	5,714
Interaction .....	33	43,445	1,317
Within tests .....	720	213,430	296

For the purpose of comparison, the variances between leaves, plants, and pots of the same test and treatment are combined to give the technical error within the tests. The variance between the tests is clearly significant, indicating the difficulty of performing comparable experiments at different times. The interaction also is significant, but not to the same degree. The largest contribution to the variance is the treatment with the different dosages of ultra-violet light.

In conformity with the probability theory of a single absorption in an ultimate virus particle causing inactivation, the survival curves decrease linearly when plotted on semi-logarithmic grid, with the exception that the observation at 300 seconds is definitely off the trend of the other observations.

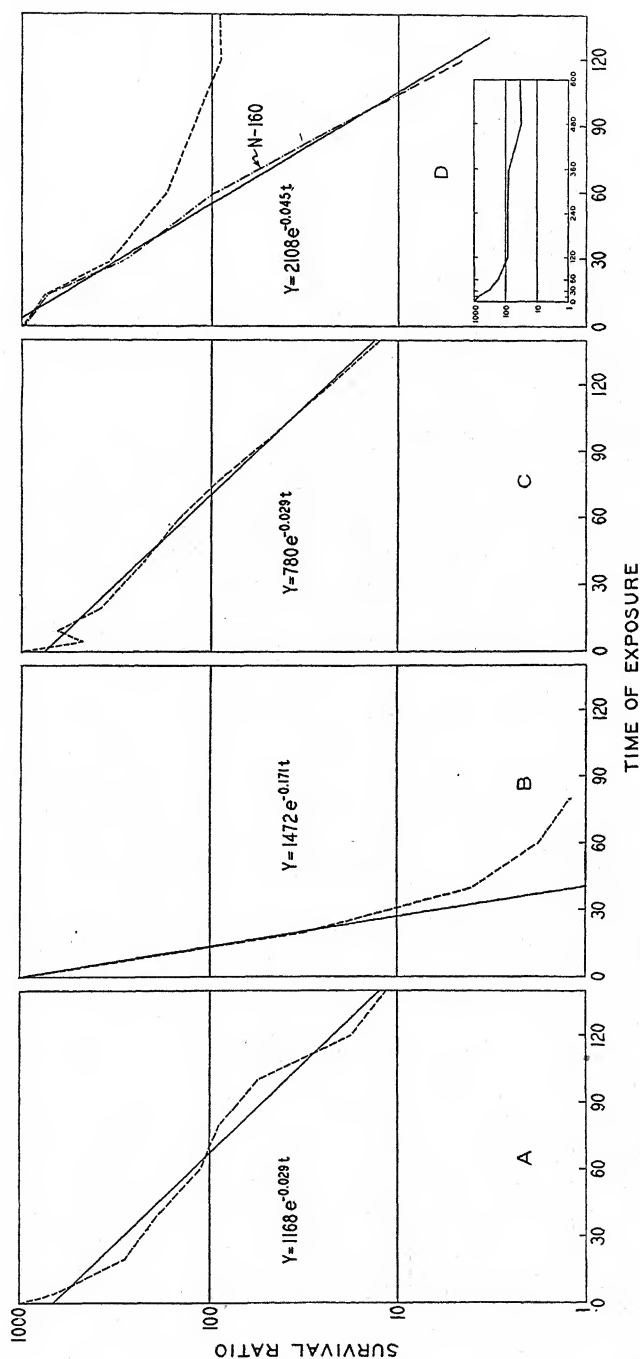


FIG. 2. Curves showing survival values for tobacco-mosaic virus exposed to ultra-violet light: A. Exposed as the filtered juice of diseased plants. B. Exposed as a solution of crystalline tobacco-mosaic virus protein. C. Exposed as a solution of crystalline protein to which juice of healthy plants was added. D. Exposed as the filtered juice of diseased plants allowed to dry down to a thin film. The line marked "N-160" represents the data plotted with 160 subtracted from each observation. The insert shows the extension of the upper curve in D.

In this particular case, the divergence is of no significance, but as it is significant in other experiments it will be discussed here. Four necrotic lesions were obtained with virus irradiated for 300 seconds, where but a fractional one should be expected. Such a result may be explained as due simply to chance. It may be explained also as due to shielding of the surviving virus particles from direct effect of the ultra-violet rays. With shielding, the curve of inactivation will drop semi-logarithmically at first, but, as exposures increase, will become concave and asymptote to a value representing the numbers of virus particles that are protected from the radiation.

To prevent such difficulties from obscuring the general trend of the ultra-violet effects, we have adopted the practice of fitting only the first points in the curves. For this particular experiment, the values that are but slightly affected by shielding are 0 to 140 seconds in tests 1 and 2, 0 to 300 seconds in test 3, and 0 to 120 seconds in test 4. The equations for the different tests are:

$$\begin{array}{ll} \text{Test 1. Lesions} = 2085 e^{-0.048t}; & \text{Test 2. Lesions} = 1210 e^{-0.032t}; \\ \text{Test 3. Lesions} = 1185 e^{-0.019t}; & \text{Test 4. Lesions} = 556 e^{-0.042t}. \end{array}$$

Comparison of the constants for tests 1 and 2 shows that they are in good agreement. The slope constant for test 3 is too low. The initial number of lesions observed in test 4 is too small, but the slope constant agrees well with those of tests 1 and 2. Since the virus samples used were taken from the same stock solution, the difference must result from the technical difficulties of the experiment and not from a difference in resistance or susceptibility of the virus.

Hollaender and Duggar (7) have performed inactivation experiments on a water-clear tobacco-mosaic virus extract mixed with *Escherichia coli* in a broth suspension. A monochromatic source of ultra-violet irradiation furnished the radiant energy. The methods by which they analyze their data are not shown, but they present one protocol. The rate of inactivation of the virus in this experiment, as calculated by the writers, follows the same type of curve as that discussed above. The slope is  $e^{-0.033t}$ . This slope is materially influenced by the wide divergence between the last observation and those that precede it, in conjunction with the fact that the time scale is logarithmic. Omitting the last observation (at 160 minutes), the slope becomes  $e^{-0.016t}$ , a slope that is in much better agreement with the remainder of the data. This slope gives 43 minutes, or 248,000 ergs, for 50 per cent inactivation of the virus. The former gives about half of these values, 21 minutes or 119,000 ergs. The nonspecific variation in the data is quite large, as in the writer's experiments. The numerical values of the slope constants cannot be compared with those obtained in the present study for two reasons: the energy values from the two sources differ in amount and in wave length and, secondly, the amount of energy absorbed in the débris is not

comparable. The fact that the form of the curve is the same as that observed in this study is the significant point.

### Crystalline Material

In order to compare the rate of inactivation of purified and nonpurified tobacco-mosaic virus samples, 4 tests were made with a solution of crystalline tobacco-mosaic protein prepared by the method of Stanley (19, 20). The concentration of virus in the solution was approximately the same as that in the nonpurified juice used in the previous experiment. Results of the 4 tests are given in table 2. They also are shown graphically in figure 2, B.

TABLE 2.—*Survival of tobacco-mosaic virus exposed to ultra-violet light. The virus was contained in a solution of crystalline virus protein*

Time of exposure	Test 1	Test 2	Test 3	Test 4	Total
0 seconds	1780 <sup>a</sup> ± 179.2 <sup>b</sup>	1901 <sup>a</sup> ± 280.3 <sup>b</sup>	1159 <sup>a</sup> ± 129.6 <sup>b</sup>	844 <sup>a</sup> ± 110.4 <sup>b</sup>	5684
5 "	718 ± 113.1	812 ± 130.4	554 ± 85.8	528 ± 93.1	2612
10 "	235 ± 33.1	320 ± 33.9	191 ± 32.5	321 ± 66.4	1067
20 "	75 ± 11.5	6 ± 2.1	44 ± 15.5	64 ± 7.7	189
40 "	5 ± 2.4	2 ± 1.4	6 ± 2.1	10 ± 3.8	23
60 "	2 ± 2.1	5 ± 2.4	0	3 ± 2.2	10
80 "	1 ± 1.0	3 ± 1.6	2 ± 1.4	1 ± 1.0	7
100 "	2 ± 1.4	2 ± 2.1	0	0	4
120 "	1 ± 1.0	8 ± 3.4	0	0	9
140 "	2 ± 1.4	3 ± 1.6	0	0	5
200 "	0	3 ± 1.6	0	0	3
300 "	0	1 ± 1.0	0	0	1

<sup>a</sup> Numbers of lesions produced in 16 bean leaves.

<sup>b</sup> Standard error of the mean multiplied by 16, the number of leaves used for test.

A comparison of the data of this table with those given in table 1 shows that purified virus is much more rapidly inactivated than nonpurified, the purified samples being virtually inactive after an exposure of from 20 to 40 seconds. Here again the survival of virus for periods longer than 40 seconds is probably due to shielding from direct effects of the ultra-violet.

The analysis of variance of the data of table 2 is given below.

	Degrees of freedom	Sum of squares	Mean square
Between ultra-violet treatments .....	11	509,422	46,311
Between tests .....	3	6,320	2,107
Interaction .....	33	5,629	171
Within tests .....	720	221,579	308

The data show a highly significant difference between the virus samples exposed to ultra-violet light for different periods of time and also a significant difference between the 4 tests. The interaction is not significant, indicating a similar trend in all 4 tests.

Using the 0 to 40 seconds' observation for tests 1, 2, and 3 and the 0 to 60 seconds' observation for test 4, the slope constants for the different tests in the experiment were calculated and are as follows:

Test 1. Lesions =  $1397 e^{-0.144t}$ ; Test 2. Lesions =  $1398 e^{-0.182t}$ ;  
 Test 3. Lesions =  $910 e^{-0.132t}$ ; Test 4. Lesions =  $915 e^{-0.108t}$ .

Although there is considerable variation between the initial number of lesions of each test, it will be noted that the slope constants for all of the tests are fairly uniform. The average value for the slope constants of the 4 tests in this experiment is about 4 times as large as the average for tests with juice of diseased plants. The difference in the rate of inactivation of virus in purified and nonpurified samples may be explained by the fact that the plant juice contains extraneous material that may absorb ultra-violet light and thus reduce the quantity that reaches the virus. This hypothesis is given further consideration in the next section of the paper.

#### Crystalline Tobacco-mosaic Virus Protein Plus Juice of Healthy Plants

If the difference in rate of inactivation of virus in purified and nonpurified preparations depends upon the presence of extraneous material in the

TABLE 3.—*Survival of tobacco-mosaic virus exposed to ultra-violet light. The virus was contained in a solution of crystalline virus protein to which filtered juice of healthy plants was added*

Time of exposure	Test 1	Test 2	Test 3	Test 4	Total
0 seconds	473 <sup>a</sup> ± 85.8 <sup>b</sup>	1302 <sup>a</sup> ± 202.9 <sup>b</sup>	837 <sup>a</sup> ± 118.2 <sup>b</sup>	1425 <sup>a</sup> ± 240.3 <sup>b</sup>	4037
5 "	386 ± 69.4	656 ± 72.0	375 ± 50.4	477 ± 99.7	1894
10 "	492 ± 59.0	698 ± 80.6	683 ± 58.6	766 ± 110.7	2639
20 "	110 ± 26.9	931 ± 118.6	216 ± 29.8	303 ± 30.9	1560
40 "	127 ± 17.8	424 ± 104.8	249 ± 43.0	126 ± 21.0	926
60 "	89 ± 18.4	389 ± 70.9	54 ± 12.0	57 ± 12.8	589
80 "	38 ± 6.9	228 ± 45.8	31 ± 11.4	39 ± 10.4	336
100 "	25 ± 6.6	115 ± 18.1	12 ± 5.6	17 ± 3.7	169
120 "	12 ± 4.5	71 ± 20.6	3 ± 3.0	7 ± 2.6	93
140 "	5 ± 2.4	33 ± 11.5	10 ± 3.8	1 ± 1.0	49
200 "	0	2 ± 1.4	0	0	2
300 "	0	3 ± 1.6	0	0	3

<sup>a</sup> Numbers of lesions produced in 16 bean leaves.

<sup>b</sup> Standard error of the mean multiplied by 16, the number of leaves used for test.



nonpurified samples, then the addition of such material should reduce or eliminate the difference between the two preparations. Tests were, therefore, made with crystalline material to which juice of healthy tobacco plants had been added. The virus concentration in this solution was the same as that in the samples used in the previous experiment, while the concentration of tobacco juice was the same as that in the nonpurified virus samples used in the first experiment. The results of 4 tests are given in table 3.

The data show that the rate of inactivation was approximately the same as that for nonpurified virus. This is shown more clearly by comparing the graphs (Fig. 2) for the two sets of data and by comparison of the slope constants for the nonpurified material with those for the purified material plus healthy juice. The constants for the latter, omitting the observations from 200 to 300 seconds for tests 1 and 2 and the observations from 140 to 300 seconds for tests 3 and 4, are:

Test 1. Lesions =  $440 e^{-0.031t}$ ;    Test 2. Lesions =  $1127 e^{-0.023t}$ ;  
 Test 3. Lesions =  $771 e^{-0.043t}$ ;    Test 4. Lesions =  $846 e^{-0.040t}$ .

The average for the 4 tests is approximately the same as the for the non-purified virus samples.

The analysis of variance of the data of table 3 is given below.

	Degrees of freedom	Sum of squares	Mean square
Between ultra-violet treatments .....	11	281,845	25,622
Between tests .....	2	27,507	9,169
Interaction .....	33	50,833	1,540
Within tests .....	720	199,941	278

The analysis shows a highly significant difference between treatments and a smaller but significant difference between tests. The interaction is likewise significant.

#### Dried Material

Since ultra-violet rays do not penetrate to any great depth of solution, it was thought that nonpurified virus might be inactivated more quickly if irradiated as the dry film left in the dish after the water had evaporated off. One-cc. samples of the 1:10 dilution of virus were placed in each dish and allowed to dry overnight, or longer, before being irradiated. Table 4 shows the results that were obtained.

When the curve for the data of table 4 is compared with that for the data of table 1 (Fig. 2), striking differences appear. The plot for the solution of virus is a straight line. The graph for virus in the dry film is far from

TABLE 4.—*Survival of tobacco-mosaic virus exposed to ultra-violet light. The virus was contained in filtered juice of diseased plants allowed to dry down to a thin film*

Time of exposure in seconds	Test 1	Test 2	Test 3	Test 4	Test 5	Total
0 .....	1706 <sup>a</sup> ± 296.8 <sup>b</sup>	1424 <sup>a</sup> ± 174.6 <sup>b</sup>	1652 <sup>a</sup> ± 187.0 <sup>b</sup>	2766 <sup>a</sup> ± 416.0 <sup>b</sup>	1943 <sup>a</sup> ± 290.4 <sup>b</sup>	9491
15 .....	1785 ± 205.0	1240 ± 149.6	1021 ± 129.9	2188 ± 272.0	966 ± 181.0	7200
30 .....	921 ± 239.8	672 ± 64.0	399 ± 67.2	797 ± 193.8	469 ± 97.1	3258
60 .....	678 ± 106.9	204 ± 18.7	214 ± 26.1	363 ± 43.8	167 ± 41.6	1626
120 .....	251 ± 39.0	147 ± 34.7	120 ± 18.9	206 ± 31.0	116 ± 21.4	840
240 .....	361 ± 80.5	135 ± 19.7	121 ± 16.8	196 ± 30.4	66 ± 13.9	879
360 .....	103 ± 29.6	149 ± 24.5	73 ± 12.8	457 ± 76.8	16 ± 6.4	798
480 .....	42 ± 10.2	74 ± 22.4	80 ± 15.0	103 ± 19.5	12 ± 3.7	311
600 .....	54 ± 17.9	87 ± 13.3	53 ± 15.5	96 ± 34.4	88 ± 18.9	378
1200 .....	5 ± 2.4	30 ± 4.6	52 ± 8.0	13 ± 3.7	10 ± 5.1	110

<sup>a</sup> Numbers of lesions produced in 16 bean leaves.

<sup>b</sup> Standard error of the mean multiplied by 16, the number of leaves used for test.

*Analysis of variance*

	Degrees of freedom	Sum of squares	Mean square
Between ultra-violet treatments .....	9	1,194,851	132,761
Between tests .....	4	56,792	14,198
Interaction .....	36	115,635	3,212
Within tests .....	750	704,555	939

straight; it is concave and asymptotes not at zero lesions but at from 100 to 200 lesions. It seems evident that the virus particles, being fixed in position with regard to the inert material, instead of circulating in a medium by means of convection currents and thus avoiding such material, are, in about 10 per cent of the instances, shielded from direct hits by the ultra-violet light. The low penetrating power of these rays consequently prevents them from inactivating this much virus. The other 90 per cent of the virus may be directly reached by the light. For comparative purposes the slopes for the initial 60 seconds of exposure to ultra-violet light are presented:

Test 1. Lesions =  $1822 e^{-0.017t}$ ;    Test 2. Lesions =  $1711 e^{-0.034t}$ ;  
 Test 3. Lesions =  $1544 e^{-0.035t}$ ;    Test 4. Lesions =  $2811 e^{-0.034t}$ ;  
 Test 5. Lesions =  $1812 e^{-0.041t}$ .

The initial lesion counts for the 5 tests are similar. The slopes of 4 of the 5 tests are comparable, that of the first test being somewhat low.

These slopes are affected by the shielding effect cited above. We wish to arrive at the actual survival ratios for the virus that is under direct exposure to the ultra-violet rather than the ratios presented above. The case may be considered as follows. The material exposed to ultra-violet light may be grouped into two parts: (A) virus that is directly exposed to irradiation and, therefore, may be inactivated by it, and (B) virus that is shielded by extraneous plant debris and is not inactivated. The number of particles in the first group may be represented by A, the amount of the shielded virus by B. If dried material is exposed for unit time the rate of inactivation may be regarded as R, R being between 0 and 1. The effects on the virus after successive units of time exposure to ultra-violet irradiation are:

Time of exposure	Virus remaining active	Virus inactivated
0	A + B	0
1	A (1-R) + B	AR
2	A (1-R) <sup>2</sup> + B	AR (1-R)
3	A (1-R) <sup>3</sup> + B	AR (1-R) <sup>2</sup>
:	:	:
:	:	:
n	A (1-R) <sup>n</sup> + B	AR (1-R) <sup>n-1</sup>

Since (1-R) is a fraction, it is evident that the curve showing the effect of the ultra-violet light will asymptote to B instead of 0, as in the previous experiments. Since R on the whole is not small compared with 1, it follows that (1-R) becomes rapidly smaller at each increase in exponent. The asymptotic value, or B, will consequently be quickly reached.

An estimate of the constants involved in the experiment may be made in different ways. Possibly the best is to estimate the value of B from the value

assumed by the curve after it has apparently arrived at the asymptotic value and then calculate the other desired quantities. The estimate of B will, of course, be influenced by the random variation inherent in the experiment. The value of B is indicated as the lower asymptote of the curve presented in figure 2, D, and is estimated at 160 particles out of a total of approximately 1900. Data showing the true survival ratios may be obtained by subtracting 160 from each of the original values. In the calculations, the data of table 4 were averaged, 160 was subtracted from each average, and the slope constant for the residual was calculated. The resulting equation is:

$$\text{Lesions} = 2108 e^{-0.045t}$$

The slope constant, 0.045, is somewhat larger, although not significantly larger, than the average of those obtained for nonpurified virus in solution and for purified virus plus healthy juice. The slope constant is, however, distinctly smaller than the average of those for the dissolved crystals. It is concluded that the rate of inactivation of dried virus exposed to ultra-violet light is essentially the same as that for nonpurified virus in solution. Unfortunately, virus purified by crystallization will not stand drying in air, so that the comparison cannot be carried further.

#### DISCUSSION

The foregoing data show that survival values for tobacco-mosaic virus exposed to radiant energy of the ultra-violet band follow the simplest of exponential curves. This type of curve is applicable to the inactivation of many living things or their genes. The survival ratios for certain bacteria, such as *Bacillus coli*, *B. aertrycke*, and *Staphylococcus aureus*, and for *Drosophila melanogaster* sperm follow this simple exponential type of curve. Radiation of greater intensity, such as X-rays, gamma rays, alpha particles, or cathode rays, also cause inactivation in a similar manner (5). The case of *Drosophila* is particularly useful because of the fact that irradiation is known to produce mutation in this insect. Irradiation of *Drosophila* sperm by X-rays of wave lengths from 2.29 Å to 0.7 Å, or less, results either in the death of these sperm, the production of lethal rearrangements in the chromatin (within the gene or the linin thread), or gene mutations. Each of these characteristic effects of the radiant energy follows the same exponential type of curve.

The best evidence available indicates that the gene is a single unit capable of reproducing itself sometimes during the cell cycle. The absorption of energy within this structure may cause alterations so extreme as to result in inactivation, or it may result in an alteration less extreme but sufficient to produce changes the effects of which are observed in somatic characters.

The fact that tobacco-mosaic virus is inactivated by ultra-violet light in a manner similar to the inactivation of genes suggests the possibility that the virus, like genes, may be capable of mutating under the stimulus of radiant energy.

#### SUMMARY

The survival values of tobacco-mosaic virus exposed to ultra-violet light follow a simple exponential curve. If we regard radiant energy as absorbed in discrete units, this curve may be obtained when one unit of energy absorbed in a virus particle is sufficient to cause its inactivation. The rate of inactivation will depend on the amount of energy incident to the virus. The data show that when the virus is most purified (in a solution of crystalline material) and the solution has least extraneous matter to absorb the energy, the rate of inactivation is greatest. Adding juice of healthy tobacco plants to purified virus lowers the rate of inactivation. The rate for the crystalline material plus juice of healthy tobacco plants is essentially the same as that for virus in juice of diseased plants. The rate of inactivation for virus in nonpurified dried juice follows essentially the same curve as that for the wet material, except that a portion of the virus fails to become inactivated even when exposed for long periods of time. This is believed to be due to the fact that dried virus particles, because of their fixed position, are sometimes overlain by other material and thus shielded from the ultra-violet light.

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# A COMPARATIVE STUDY OF BACTERIUM TABACUM WOLF AND FOSTER AND BACTERIUM ANGULATUM FROMME AND MURRAY<sup>1</sup>

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## INTRODUCTION

The tobacco wildfire organism, *Bacterium tabacum* Wolf and Foster<sup>2</sup> (16), was first described in 1917 from North Carolina. Two years later, in Virginia, Fromme and Murray (4) described an organism causing angular leaf spot or blackfire of tobacco, which they named *Bacterium angulatum*.<sup>3</sup> Although the earlier and subsequent studies of the two organisms involved showed that they were on the whole similar, the two diseases were accepted by other American pathologists as being pathologically distinct and essentially unrelated etiologically. Both diseases were later found widespread in the tobacco-growing districts of the United States, and also appeared in several foreign countries. There appeared, however, to be no indication in the local or widespread distribution of the two diseases that would suggest any relationship between them.

When Stapp (13), working in Germany, announced in 1930 that the bacteria causing wildfire and those causing angular leaf spot were identical, it naturally stimulated a new interest in these diseases, even though much skepticism remained as to the validity of the conclusion. The marked difference in symptoms induced on tobacco by *Bacterium tabacum* and *Bact. angulatum*, and the apparent difference in virulence of the two organisms should not be overlooked. In order advantageously to approach these questions of virulence and symptomology, it is obvious that detailed descriptions of both organisms be made from cultures of known purity and pathogenic behavior. The purpose of the present paper is to furnish a more complete bacteriological foundation for the comparison of the two organisms in question, and at the same time to recognize the significance of such pathological differences as may exist or may be induced.

<sup>1</sup> The writer is greatly indebted to Professor James Johnson, under whose direction this work was conducted, and to Professor A. J. Riker for criticism and suggestions throughout the course of the investigation.

<sup>2</sup> Synonyms: *Phytomonas tabaci* (Wolf and Foster) Bergey et al. *Pseudomonas tabaci* (Wolf and Foster) Stapp.

<sup>3</sup> Synonyms: *Phytomonas angulata* (Fromme and Murray) Bergey et al. *Pseudomonas angulata* (Fromme and Murray) Stapp.

## REVIEW OF LITERATURE

The first comparison made between *Bacterium tabacum* and *Bact. angulatum* was reported by Fromme and Murray in 1919 (4). These workers presented in tabulated form 5 contrasting features found to exist between the newly described angular-leaf-spot organism and the wildfire organism, as characterized by Wolf and Foster (17) in 1918. Fundamental differences relative to the size of the two organisms, number of flagella, rate of gelatin liquefaction, production of acids with certain carbon sources, and type of growth in fermentation tubes were reported in this early publication as distinguishing features.

The size of the wildfire organism, according to Wolf and Foster (17), varies from  $2.4-5 \times 0.9-1.5 \mu$ . Slagg (11) 1921, Anderson and Chapman (1) 1921, and Clinton and McCormick (3) 1922, agree in their measurements of the wildfire organism and they report that *Bacterium tabacum* varies from  $1.4-2.8 \times 0.5-0.8 \mu$ . Fromme and Murray (4) state that the angular-leaf-spot organism is a short rod with rounded ends,  $2.0-2.5 \times 0.5 \mu$  in size.

The wildfire organism was described by Wolf and Foster (17) as possessing a single polar flagellum. Subsequent work, however, by Slagg (11), Anderson and Chapman (1), Clinton and McCormick (3), Johnson and Fracker (6), and Stapp (13) among others, has failed to corroborate Wolf and Foster's results, and these investigators showed a tuft of 1 to 6 polar or bipolar flagella to be present. Fromme and Murray (4) state that *Bacterium angulatum* is motile by means of a tuft of from 3 to 6 polar flagella.

Wolf (14) 1922 reported that comparative cultural studies of the wildfire and angular-leaf-spot organisms revealed constant differences that did not lend themselves readily to description. This investigator separated the two organisms readily on the basis of the ability of *Bacterium tabacum* to ferment galactose and mannitol, neither of which was utilizable by *Bact. angulatum*. Previous cultural studies (17) could not be corroborated. Wolf (15) also concluded that the colonies of the two organisms differed sufficiently, even in the same Petri-dish culture, to be readily distinguishable.

A serological relationship was found by St. John-Brooks, Nain, and Rhodes (10) to exist between *Bacterium tabacum* and *Bact. angulatum* as well as between several other species of green fluorescent bacteria. These workers arbitrarily carried out the agglutination tests with a serum dilution of 1:100 and did not cover the entire range of the fairly potent antisera that were used in their studies. Because of insufficient dilution of the antisera, these investigators could not determine how closely the organisms were related; their results merely indicate that a relationship exists.



An extensive comparative study of 18 bacterial strains that cause leaf-spot diseases of tobacco has more recently been presented by Stapp (13). With but 2 exceptions, this worker did not indicate whether the strains of organisms used were of angular-leaf-spot or wildfire type, and merely used arbitrary designations. He reports, however, that *Bacterium tabacum* and *Bact. angulatum* are indistinguishable by the agglutination and precipitation tests, and he concludes that the organisms are identical chiefly on the basis of these reactions. Stapp proposes to place the angular-leaf-spot organism in the same species with the previously named wildfire organism.

These papers are obviously not in accord on various questions important for studies of pathogenicity. In no case were cultures of single-cell origin employed, which raises some doubt as to the purity of some of the cultures used. In certain of these studies (*e.g.*, by Stapp, 13) the pathogenic characters of the cultures used were either negative or questionable. These considerations in particular made imperative a better foundation through detailed bacteriological studies on single-cell cultures of known pathogenic behavior.

#### MATERIALS AND METHODS

The chief strains used in the experimental work were obtained from widely separated fields in Southern Wisconsin. Six strains of the angular-leaf-spot organism and 4 strains of the wildfire organism, respectively, were the progeny of single bacterial cells. Single-cell isolations were made according to the method described by Wright *et al.* (18). Several cultures of each organism, not of single-cell origin, were used in the various tests when needed. For the sake of convenience, the angular-leaf-spot strains were designated by the use of numbers, and the wildfire strains by letters. Strains 1, 2, 3, 4, 5, and 6 and A, B, C, and D were of single-cell origin. All cultures were carried on potato-dextrose agar, were transferred at monthly intervals, and were stored at 8° C. The pathogenicity of the cultures was determined by inoculation of tobacco. All cultures were found to be virulent and gave rise to typical symptoms in the host plant.

The bacteriological and serological work reported in this paper was performed according to the methods reported by the Society of American Bacteriologists (12). Special technique used in the comparative studies will be referred to in the respective sections in which the subjects are discussed. Unless otherwise noted all experiments were performed 3 times.

The tobacco (*Nicotiana tabacum* L.) plants used in the pathological studies were of a pure-line strain of Havana seed locally known as Havana No. 38. All plants were grown in compost soil in 4-inch pots, and were inoculated when in the 4- or 5-leaf stage. Following inoculation, the plants were placed in a large moist chamber in which the humidity and tempera-

ture were controlled. The temperature used was usually 25 to 28° C., and the relative humidity of the air, 90 to 95 per cent. In order to determine the relative toxin-producing ability of the various wildfire cultures, inoculations to tobacco plants were made by means of needle puncture. The non-toxin-producing cultures of *Bacterium tabacum* and the angular-leaf-spot bacteria were inoculated to plants by means of an atomizer so as to facilitate stomatal infection. The plants were placed in the control chamber for about eight hours before inoculation, and kept in the chamber for about twenty-four hours after inoculation.

#### EXPERIMENTAL RESULTS

##### Morphological Studies

The questions that arose in the earlier literature pertaining to the number of flagella and the relative sizes of the organisms have been reviewed in a previous section. *Bacterium tabacum* and *Bact. angulatum* are now considered; by nearly all investigators, to be morphologically indistinguishable. These findings are in agreement with those obtained by the writer and are summarized here: Size,  $0.5 \times 2-2.5 \mu$ ; flagella (Casares-Gils' stain), 1-6 polar and bipolar; spores (Dorner's method), absent; capsules (Ginn and Hiss methods), absent; Gram stain (Kopeloff's modification), negative; acid-fast reaction, not acid-fast.

##### Cultural, Physical and Biochemical Studies

*Colony Type.* According to Wolf (15), it is possible to distinguish colonies of *Bacterium tabacum* from those of *Bact. angulatum*. Stapp (13), however, found variation in the colonies within individual strains, and concludes that definite distinction between the two species on this basis is impossible. While it is not unlikely that even minor differences in the media, and other conditions of environment may account for the variations in behavior observed, it is obvious that much significance cannot be attached to results that are difficult or impossible to duplicate. It has been the writer's observation that no significant differences in colony characteristics appear to exist between *Bact. tabacum* and *Bact. angulatum* on any of the media used in the present studies. In agreement with Stapp, it was found rather that the variation between the strains of each species was greater than that between the species themselves. Spontaneous dissociation into rough, intermediate, and smooth colonies was a fairly common phenomenon with both *Bact. tabacum* and *Bact. angulatum*.

The development of colonies by both *Bacterium tabacum* and *Bact. angulatum* was observed on nutrient agar composed of 3.0 grams beef extract, 5.0 grams peptone, 15 grams agar, and 1000 cc. distilled water. The reac-

tion of the medium was adjusted to pH 7.0. Dilution plates were poured, using several strains of each organism, and only Petri dishes containing approximately 20 well-distributed colonies were used for observation. It was found that, after a 20- to 24-hour incubation period at 26° C., small glistening colonies began to appear; and, at the end of 72 hours, many had reached a diameter of 3 millimeters. A typical colony, upon macroscopic examination, was found to be round, white, slightly raised in elevation, and to have a translucent, rather well-defined edge and an opaque center. Microscopic examination with the low power objective revealed an entire to a slightly undulate well-defined margin. The colony was found to be homogeneous and nongranulate. The center of the colony was dull yellow but gradually became lighter toward the outside, and a clear translucent margin was present.

*Gelatin Liquefaction.* It generally is agreed that gelatin is liquefied by both organisms, although *Bacterium tabacum* has been reported to liquefy gelatin more slowly than *Bact. angulatum*. Stapp (13) considers that, because an American culture of *Bact. tabacum* liquefied gelatin slowly, it is less closely related to the European strains of the wildfire organisms than was an American culture of *Bact. angulatum*, which acted more rapidly.

The writer has been able to demonstrate, by means of a rather simple experiment, that the ability of these two organisms to liquefy gelatin varies with frequency of transfer. Four angular-leaf-spot cultures and 3 wildfire cultures, all of which were active gelatin liquefiers, were transferred on potato-glucose-agar slants at weekly and monthly intervals for a period of 1½ years. About 30 tubes of the nontransferred original cultures of each strain were kept at 8° C., together with the weekly and monthly cultures. It was found that the cultures transferred at weekly intervals showed a gradual but definite decrease in ability to liquefy gelatin, and, by the end of the 18-month period, had lost this property entirely. The cultures that were not transferred during the course of the experiment retained their original ability to liquefy gelatin; while those transferred at monthly intervals liquefied gelatin more slowly than did the original strains, but they did not lose the property entirely.

There was no difference found in the rate and type of liquefaction with *Bacterium tabacum* and *Bact. angulatum* when rapidly liquefying cultures of both organisms were used.

*Dye Absorption.* Several workers, including Kellerman (8), Riker *et al.* (9), and Hendrickson *et al.* (5), have used dyes successfully in the differential studies of various bacteria. Sixteen representative dyes selected from the various dye groups were used in a preliminary study at a concentration of 1:10,000 in yeast-water-mannitol agar. The following dyes were used:

*Azo Dyes*: Bismarck brown, Methyl orange; *Quinone-imide Dyes*: Nigrosin, Safranin, Thionin; *Xanthene Dyes*: Eosin, Flourocein; *Compound Dyes*: Dahlia eosin; *Natural Dyes*: Orcein; *Phenol Methane Dyes*: Anilin blue, Acid fuchsin, Basic fuchsin, Crystal violet, Dahlia, Night blue, Rosanilin.

The various strains of both groups of organisms were studied as giant colonies in plate culture. The plates were incubated for 14 days at room temperature. There was no bacteriostatic effect produced at the concentration of the dyes used, and no differential dye absorption was observed. In this test it was again found that the colonies, both of the various wildfire strains and of the various angular-leaf-spot strains differed more among themselves than the colonies of the wildfire strains differed from those of the angular-leaf-spot strains.

Several other cultural and diagnostic studies have been applied in a comparative study of 5 strains of *Bacterium tabacum* and 5 strains of *Bact. angulatum*. Since the reaction of the bacteria to the various tests was found to be identical, a summary of these is presented here: Bouillon, heavy clouding, no pellicle; litmus milk, clearing, and casein precipitated; water soluble starch, no diastatic action; nitrate broth, nitrate not reduced; sodium selenite-agar medium, no growth; Loeffler's blood agar, liquefaction after 3 weeks; potato, rather abundant cream-color growth; tryptophane broth for indol test (Böhme method), no indol produced; minimum growth temperature, about 4° C.; optimum growth temperature, 24–28° C.; maximum growth temperature, about 38° C.; thermal death point (10-minute exposure) 49–51° C.

*Utilization of Carbon Sources.* Wolf (14) claimed to be able to distinguish between *Bacterium angulatum* and *Bact. tabacum* because of the ability of the latter to ferment galactose and mannitol, neither of which was utilizable by *Bact. angulatum*. Stapp (13), on the other hand, was unable to find sufficient difference between the various strains of the two organisms to be able to differentiate them with certainty.

Because of these apparent discrepancies that exist in some of the previous work, a study on the availability of various carbon sources was undertaken. The basic medium used in the present investigation was composed of the following materials: magnesium sulphate ( $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ) 0.2 gram; sodium chloride ( $\text{NaCl}$ ) 0.2 gram; calcium chloride ( $\text{CaCl}_2$ ) 0.1 gram; dipotassium phosphate ( $\text{K}_2\text{HPO}_4$ ) 0.2 gram; a carbon source 5 grams; distilled water 900 cc.; and yeast-water extract 100 cc.

The yeast-water extract was prepared by autoclaving 100 grams of starch-free yeast in 1000 cc. of distilled water. Only a slight trace of growth was made by the bacteria in the basic medium into which no source of carbon had been introduced.

The carbon source and the other ingredients were made up in double strength, and sterilized separately on 3 successive days at 10 lbs.' pressure for 10 minutes. The pH was adjusted to approximate neutrality, and the materials in the flasks were mixed and tubed under aseptic conditions. After an incubation period of 1 week to insure sterility, the tubes were seeded with a standardized aqueous suspension of a 48-hour potato-glucose-agar culture of the desired organisms. The cultures were incubated at room temperature for 14 days, at which time the pH readings were made. The hydrogen-ion concentration was measured electrometrically by means of the quinhydrone-electrode method. No gas production was observed in any of the media.

TABLE 1.—Range of pH and relative growth of six strains of *Bacterium angulatum* and five strains of *Bacterium tabacum* in yeast-water-mineral-salts media containing various carbon sources

Carbon sources added	pH readings					Relative growth <sup>a</sup>	
	Uninoculated check	Range	Average	Range	Average	<i>Bacterium angulatum</i> (6 strains)	<i>Bacterium tabacum</i> (5 strains)
		<i>Bacterium angulatum</i> (6 strains)		<i>Bacterium tabacum</i> (5 strains)			
Glucose .....	7.2	6.1-7.1	6.6	6.1-6.7	6.3	+++	+++
Galactose .....	7.2	5.9-7.2	6.7	5.2-6.7	6.2	+++	+++
Levulose .....	7.1	7.1-7.5	7.4	7.3-7.5	7.4	+++	+++
L-Arabinose...	7.2	4.9-7.5	5.9	5.1-5.4	5.5	+++	+++
Xylose .....	7.1	5.1-7.0	5.9	5.1-5.8	5.2	++	+++
Sucrose .....	7.2	5.8-7.3	6.8	6.5-6.8	6.6	++	++
Maltose .....	7.2	7.7-8.0	7.9	7.8-8.0	7.9	±	±
Lactose .....	7.2	7.7-8.0	7.8	7.8-8.0	8.0	±	±
Dextrin .....	7.2	7.9-8.1	8.0	7.9-8.1	8.0	±	±
Inulin .....	7.2	7.8-8.1	8.0	7.9-8.0	7.9	±	±
Pectin .....	7.0	7.2-7.3	7.3	7.2-7.3	7.3	++	++
Mannitol .....	7.1	7.3-7.6	7.5	7.3-7.5	7.4	+++	+++
Glycerol .....	7.1	7.5-7.6	7.5	7.4-7.5	7.5	+++	+++
None .....	7.0	7.9-8.0	8.0	8.0-8.0	8.0	±	±

<sup>a</sup> +++ Good growth.

++ Moderate growth.

± Trace of growth.

The results given in table 1 clarify the carbon metabolism of these organisms and show that they were not differentiated by the use of the various representative carbon sources in yeast-water-mineral-salts media. Considerable variation was noted in the amount of acid production by various strains in media containing glucose, galactose, l-arabinose, xylose, and sucrose. For example, Strains 1 and 5 had final readings of 7.0 to 7.5 in media containing glucose, l-arabinose, xylose, and sucrose. This variation might explain certain discrepancies in earlier reports.

*Utilization of Nitrogen Sources.* The availability of various representative nitrogen sources was determined in further comparative studies of the two organisms. The media for studying the utilization of the nitrogen-containing compounds were made by substituting these materials for the yeast water in the basic medium. Mannitol was used as the source of carbon. Concentrations of 0.5 per cent by weight of the nitrogenous substances were used in all of the media except in the case of l-cystine, which was employed in 0.1 per cent concentration. Preparation of the media, seeding, and incubation were the same as described for the utilization of the carbon sources. The experiment was performed twice, and a summary of the results is given in table 2.

TABLE 2.—*Range of pH and relative growth of six strains of *Bacterium angulatum* and five strains of *Bacterium tabacum* in mannitol-mineral-salts medium containing various nitrogen sources*

Nitrogen sources added	pH readings					Relative growth <sup>a</sup>	
	Uninoculated check	Range	Average	Range	Average	<i>Bacterium angulatum</i> (6 strains)	<i>Bacterium tabacum</i> (5 strains)
		<i>Bacterium angulatum</i> (6 strains)		<i>Bacterium tabacum</i> (5 strains)			
Ammonium sulphate .....	6.5	4.6-4.7	4.6	4.6-4.9	4.8	+	+
Potassium nitrite .....	7.5	7.5-7.5	7.5	7.5-7.5	7.5	-	-
Potassium nitrate .....	7.2	6.3-6.8	6.5	6.4-6.9	6.6	+++	+++
Cystine .....	7.2	5.3-5.9	5.7	5.5-6.4	6.0	++	++
Glutamic acid .....	7.2	6.1-6.9	6.4	6.4-6.8	6.6	+++	+++
Glycine .....	7.0	5.8-6.2	6.1	5.8-7.4	6.4	++	+
Succinimide .....	6.4	6.2-6.8	6.6	6.4-7.6	6.8	++	+++
Oxamide .....	6.7	4.5-4.8	4.7	4.5-4.7	4.6	++	+++
Acetamide .....	6.9	5.9-7.2	6.4	6.2-6.9	6.7	++	++
Urea .....	7.5	8.7-8.8	8.8	8.8-8.8	8.8	++	++
N-free check	7.0	7.0-7.5	7.0	7.0-7.0	7.0	-	-

<sup>a</sup> +++ Good growth.

++ Moderate growth.

+ Slight growth.

- No growth.

These results clarify the nitrogen metabolism of the organisms and indicate that the various nitrogen sources employed do not serve to differentiate them.

#### Serological Studies

In addition to the morphological, cultural, and physiological studies, fairly comprehensive comparative serological tests were made with *Bac-*

*terium angulatum* and *Bact. tabacum*. The agglutination, agglutinin absorption, and precipitation tests were used in an attempt to determine the relationship of the two organisms. St. John-Brooks, Nain, and Rhodes (10), as well as Stapp (13), have pointed out in previous work that a close serological affinity exists between the two bacterial types. Stapp contends that when precipitation tests are positive, identity of species is confirmed, even though cultural and physiological differences exist; he, therefore, regards *Bacterium tabacum* and *Bact. angulatum* as identical organisms.

*Agglutination Tests.* Agglutination tests were made with 5 single-cell strains of the angular-leaf-spot organism and 5 strains of the wildfire organism, four of which were of single-cell origin. Normal agglutinins for *Bacterium angulatum* and *Bact. tabacum* were not found to be present in sera of the rabbits used.

The immune sera were prepared from 4 rabbits, 2 of which were injected with single-cell strains of the angular-leaf-spot organism and 2 with single-cell strains of the wildfire organism.

Cultures of the bacteria for immunization of the rabbits and for the antigens in the serological tests were grown on neutral potato-glucose-agar slants for 24 hours. Suspensions of the bacteria for immunization purposes were made up in 0.85 per cent NaCl solution by washing the bacterial growth free from the agar, shaking gently to obtain a uniform suspension, and then filtering through a fine-grade filter paper. The suspensions in each case were standardized to a turbidity that would conceal a 2 mm. loop made of No. 26 B. and S. gauge wire at a depth of 12 mm. Care was taken to use only the "smooth" form of the organisms.

Rabbits were injected intravenously at intervals of 4 to 5 days until 5 injections had been made, using successively greater amounts of suspension. The amounts of injections were, respectively: 0.5 cc., 1 cc., 1.5 cc., 3 cc., 5 cc. Ten days after the last injections, the rabbits were bled from the ear vein, and, since the titre was found to be sufficiently high in the preliminary trials, enough blood was drawn for the final agglutination tests. After coagulation of the blood, the serum was removed and preserved for subsequent tests.

The macroscopic agglutination test was used and dilutions of the antisera ranged from 1-50 to 1-12,800. The results with antisera from Strains A, C, 1 and 2, respectively, were all similar and are represented by the data in table 3. These results showed that the organisms are indistinguishable by the macroscopic agglutination test.

*Agglutinin Absorption.* The antisera used in this work were the same as those employed in the agglutination test, and had a titre of about 1-5000.

The minimum absorbing dose of the antigen was determined by dividing the homologous bacterial suspensions into 5 portions and adjusting them to

TABLE 3.—Representative data on agglutination tests with cultures of *Bacterium tabacum* and *Bacterium angulatum*<sup>a</sup>

Antigen	Strain	Dilutions <sup>b</sup> of serum from rabbit immunized with <i>Bacterium tabacum</i> Strain C						
		1-400	1-800	1-1600	1-3200	1-6400	1-12,800	s.s.
<i>Bacterium tabacum</i>	A	++++	++++	+++	+	-	-	-
	B	++++	++++	++++	++	+	-	-
	C	++++	++++	+++	+	-	-	-
	D	++++	++++	+++	++	-	-	-
	E	++++	++++	+++	++	+	-	-
<i>Bacterium angulatum</i>	1	++++	++++	+++	+	-	-	-
	2	++++	++++	+++	++	+	-	-
	3	++++	+++	++	++	-	-	-
	4	++++	++++	+++	++	+	-	-
	5	++++	++++	+++	++	+	-	-
		Dilutions <sup>2</sup> of serum from rabbit immunized with <i>Bacterium angulatum</i> Strain 1						
<i>Bacterium angulatum</i>	1	++++	++++	+++	++	-	-	-
	2	++++	++++	++++	++	-	-	-
	3	++++	++++	++	+	-	-	-
	4	++++	++++	++++	+++	+	-	-
	5	++++	++++	++	+	-	-	-
<i>Bacterium tabacum</i>	A	++++	++++	++	+	-	-	-
	B	++++	++++	++++	++	+	-	-
	C	++++	++++	++	+	-	-	-
	D	++++	++++	+++	++	-	-	-
	E	++++	++++	+++	+++	+	-	-

<sup>a</sup> Results similar to those given in this table were secured with serum from rabbits immunized, respectively, against Strains A and 2.

<sup>b</sup> Dilutions 1-50, 1-100, and 1-200 yielded the same results as did the 1-400 dilution.

++++ Very heavy agglutination.

+++ Heavy agglutination.

++ Moderate agglutination.

+ Slight agglutination.

- No agglutination.

different densities varying from 1 to 5 on the McFarland nephelometer scale. One part of nondiluted antiserum was added to 39 parts of each of the 5 antigen suspensions, giving a serum dilution of 1-40 in each case. The tubes were incubated at 37° C. for 2 hours, centrifuged, and the supernatant liquid removed to clean centrifuge tubes. Enough new bacteria from the packed centrifuge sediment of a broth culture were added to bring the respective tubes to the desired turbidity. The tubes were again permitted to incu-



bate at 37° C. for 2 hours, and the above procedure was repeated a third time, after which the tubes were allowed to stand in a refrigerator at 8° C. overnight. After the third absorption, the supernatant liquid was tested for its ability to agglutinate homologous strains of the organism. In testing strains of the other bacterial types, a slightly greater turbidity than that found necessary for the minimum absorbing dose of the homologous organism was used. This was done to exclude minor variations that are inherent in the method. The agglutination tests were set up covering a range of dilutions from 1-40 to 1-10,240. In addition to strains of *Bacterium angulatum* and *Bact. tabacum*, the A<sub>6</sub> strain of *Bact. tumefaciens* (Smith and Town.) was used as a check organism. Nontreated diluted serum was carried through the same procedure as a control.

In order to establish the true relationship of the two organisms in question, it was essential to determine not only whether the wildfire antiserum was exhausted of agglutinins for *Bacterium tabacum* by both the wildfire and angular-leaf-spot organisms, but also whether the angular-leaf-spot antiserum was exhausted of agglutinins for *Bact. angulatum* by both the angular-leaf-spot and wildfire organisms. In other words, it was essential to use the reciprocal agglutinin absorption test.

The results shown in table 4 indicate that *Bacterium tabacum* and *Bact. angulatum* are identical by this test and are not related to *Bact. tumefaciens*. Similar results, which are omitted because of their volume, were obtained when *Bact. tabacum* Strain A antiserum was absorbed with Strains A and C of *Bact. tabacum*, Strains 1 and 4 of *Bact. angulatum*, and the A<sub>6</sub> strain of *Bact. tumefaciens*, respectively, and the absorbed antiserum titrated in each case against Strains A, C, 1, 4, and A<sub>6</sub>.

*Precipitation Test.* The quantitative precipitation test also was used to study the serological relationship of the angular leaf-spot and wildfire organisms.

The bacterial precipitinogens were prepared by growing 4 cultures, 2 single-cell strains of the angular-leaf-spot organism and 2 single-cell wildfire strains, in neutral bouillon for 5 weeks and then filtering them through Berkefeld filters until the filtrates were clear and sterile.

The immune sera were prepared by injecting the agglutinin rabbits 4 additional times with living cultures of the respective organisms. The injections were made at intervals of 4 to 5 days and the rabbits were bled 10 days after the 9th injection had been administered.

The test was set up by introducing into each of 9 small test tubes 0.5 cc. of antiserum in dilutions ranging up to 1-10,000 and 0.5 cc. of undiluted precipitinogen. The 10th tube consisted of 0.5 cc. saline solution and 0.5 cc. antigen, which was used as a control.

TABLE 4.—Representative data on agglutinin absorption tests in which *Bacterium angulatum* Strain 1 antiserum was absorbed with *Bacterium tabacum*, *Bacterium angulatum*, and *Bacterium tumefaciens*. The absorbed antiserum was titrated against strains of *Bacterium tabacum*, *Bacterium angulatum*, and *Bacterium tumefaciens*<sup>a</sup>

Serum from rabbit immunized with <i>Bacterium angulatum</i> Strain 1	Strain number	Dilutions of antiserum <sup>b</sup>					
		1-320	1-640	1-1280	1-2560	1-5120	1-10, 240
<i>Bacterium angulatum</i> Strain 1 antiserum absorbed with <i>Bacterium angulatum</i> Strain 1 <sup>c</sup> Absorbed serum titrated against	1	—	—	—	—	—	—
	4	—	—	—	—	—	—
	C	—	—	—	—	—	—
	A	—	—	—	—	—	—
<i>Bacterium angulatum</i> Strain 1 antiserum absorbed with <i>Bacterium tabacum</i> Strain C <sup>c</sup> Absorbed serum titrated against	C	—	—	—	—	—	—
	A	—	—	—	—	—	—
	1	—	—	—	—	—	—
	4	—	—	—	—	—	—
<i>Bacterium angulatum</i> Strain 1 antiserum absorbed with <i>Bacterium tumefaciens</i> Strain A <sup>c</sup> Absorbed serum titrated against	A <sub>0</sub>	—	—	—	—	—	—
	1	+++	++	+	—	—	—
	C	+++	++	+	—	—	—
<i>Bacterium angulatum</i> Strain 1 antiserum not ab- sorbed with any organisms Unabsorbed serum titrated against	<i>Bacterium tabacum</i>	+++	+++	++	+	—	—
	<i>Bacterium angulatum</i>	+++	+++	++	++	—	—
	<i>Bacterium tumefaciens</i>	—	—	—	—	—	—

<sup>a</sup> Similar results were obtained when *Bacterium angulatum* Strain 1 antiserum was absorbed, respectively, with *Bacterium angulatum* Strain 4 and *Bacterium tabacum* Strain A and the absorbed serum titrated against strains A, C, 1, 4.

<sup>b</sup> Dilutions 1-40, 1-80, and 1-160 yielded the same results as did the 1-400 dilution.

<sup>c</sup> Three absorptions were made with antigen suspensions corresponding in turbidity to the No. 4 tube in the McFarland nephelometer series.

The test tubes were allowed to incubate at room temperature for 1 hour, readings being made at 15-minute intervals. A comparison of the precipitates in the respective dilutions indicated the relationship, and representative data are given in table 5. This test confirms earlier evidence that these organisms are serologically identical.

TABLE 5.—Representative data on precipitation tests with cultures of *Bacterium angulatum* and *Bacterium tabacum* in which dilutions of antiserum are titrated against constant amounts of antigen<sup>a</sup>

Antiserum and antigen	Strain	Dilutions <sup>b</sup> of antiserum					s.s.
		1-100	1-500	1-1000	1-5000	1-10,000	
<i>Bacterium tabacum</i> A antiserum							
<i>Bacterium tabacum</i> antigen	C	+	+	-	-	-	-
<i>Bacterium tabacum</i> "	A	+	+	-	-	-	-
<i>Bacterium angulatum</i> "	1	+	-	-	-	-	-
<i>Bacterium angulatum</i> "	2	+	+	-	-	-	-
<i>Bacterium angulatum</i> 2 anti-serum							
<i>Bacterium angulatum</i> antigen	1	+	-	-	-	-	-
<i>Bacterium angulatum</i> "	2	+	-	-	-	-	-
<i>Bacterium tabacum</i> "	A	+	-	-	-	-	-
<i>Bacterium tabacum</i> "	C	+	-	-	-	-	-

<sup>a</sup> Results similar to those given in this table were secured with serum from rabbits immunized, respectively, against Strains 1 and C.

<sup>b</sup> Dilutions 1-1, 1-2, 1-5, 1-10, and 1-50 yielded positive results throughout.

### Pathological Observations

*Comparison of Symptoms.* Both wildfire and angular leaf spot of tobacco are chiefly leaf diseases, though infection may occur on other aerial parts of the plant. Plants may become infected at any stage of their growth, ranging from seedlings soon after germination to green, almost mature seed pods. As a rule, young succulent tissues are more severely affected than older, less succulent tissues. The wildfire organism is more destructive, however, under essentially all conditions than is the angular-leaf-spot organism. Wildfire, may for example, kill out large areas of young plants in commercial seedbeds, whereas angular leaf spot has never been known to cause such destruction. A limited amount of killing of very young seedlings by *Bacterium angulatum* has been observed under experimental conditions. The virulent nature of the wildfire organism is apparently due largely to the toxin, which may readily become systemic, especially in young plants, hence reaching the bud and either killing the young tissues or reducing the normal rate of growth. Evidently, because of the absence of toxin, angular leaf spot

is in most respects a much less serious and less feared disease than is wildfire. Perhaps, as a consequence of this, it has become distinctly more widespread than wildfire, being almost coexistent with tobacco culture in Wisconsin, whereas wildfire is comparatively rare. As suggested in the discussion of this paper, this relative relation may, nevertheless, be interpreted otherwise.

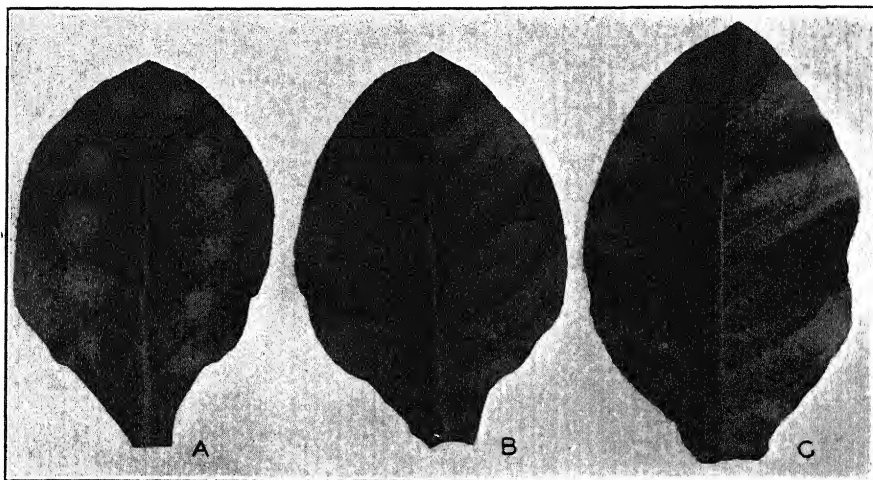


FIG. 1. Stages in the attenuation of the toxin-producing ability of *Bacterium tabacum*, as shown by the needle method of inoculation. A. Halos produced by an unattenuated strain. B. Halos produced by a partially attenuated strain. C. Results of inoculation with a completely attenuated strain. Needle inoculation yields only inconspicuous necrotic symptoms with this strain.

The chief difference between angular-leaf-spot and wildfire diseases evidently lies in the "typical" symptoms produced, as a consequence of differences in toxin present. The wide halo formed around the point of infection in wildfire (Fig. 1, A) is dependable for positive diagnosis of this disease, although *Bacterium tabacum* may cause necrosis without the halo being present, as in the "epidemic" form of the disease described by Clayton (2). Though the typical incipient necrotic lesions (*i.e.*, without halo) formed by either organism (Figs. 2 and 3) may not differ appreciably, the "epidemic" forms of the two diseases are often easily distinguishable on the basis of form and color of the necrotic area. The lesions of angular leaf spot or blackfire as the names signify are commonly angular and darker than those resulting from wildfire. It has been shown by Clayton (2) that the "epidemic" forms of the two diseases are commonly closely related to the water-soaked areas resulting from beating rain. In the instance of wildfire, the organism spreads so rapidly and destructively through the water-soaked tissue that general necrosis precedes the formation of toxin and its diffusion into sur-

rounding tissue that is not water-soaked, as is the case of the more localized halo form of the disease. This interpretation of disease expression suggests that, aside from toxin production, the differences in shape and color of the necrotic areas, as well as virulence of the diseases in question, apparently must be accounted for on the basis of inherent differences in the parasites themselves.

*Loss of Toxin Production by Bacterium tabacum.* Earlier investigators (7) recognized the fact that *Bact. tabacum* may lose its ability to produce

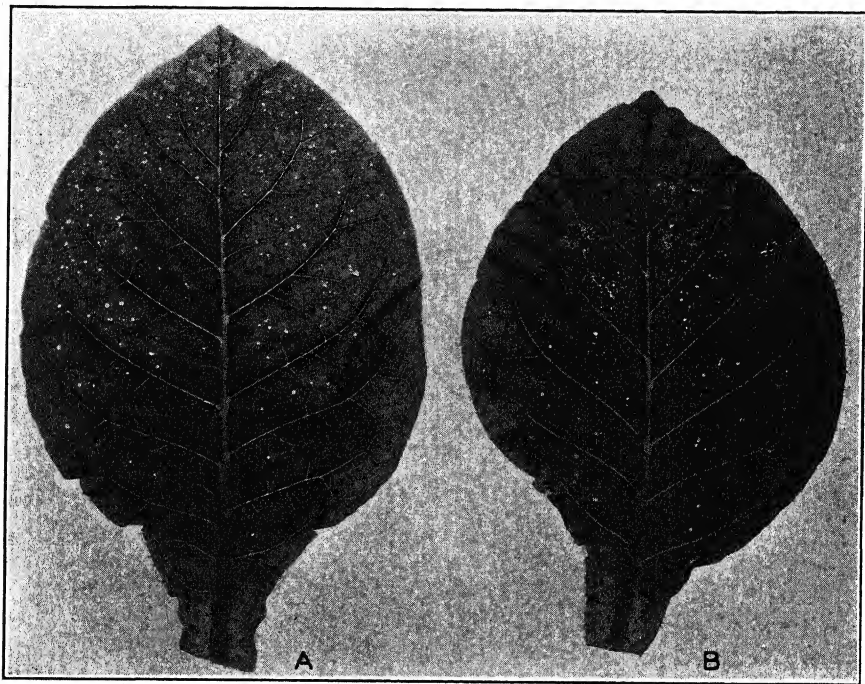


FIG. 2. Comparison of symptoms produced by *Bacterium angulatum* and a non-toxin producing culture of *Bact. tabacum*, by spray inoculation. A. Tobacco leaf inoculated with *Bact. angulatum*. B. Tobacco leaf inoculated with non-toxin producing culture of *Bact. tabacum*. Note similarity to A.

toxin when carried in culture for prolonged periods of time. These workers apparently associated loss of toxigenicity with loss of virulence and, therefore, considered the nontoxigenic wildfire organisms to be nonpathogenic, as well. Since virulence, in its broadest sense, does not consist of toxin-producing ability alone, but involves also the aggressiveness or invasive power (which refers here to the ability of bacteria to necrotize tissue) of an organism, both of these factors must be taken into consideration when the pathogenicity of a culture is studied.

A systematic attempt, therefore, was made to attenuate the toxicity factor of 3 single-cell strains of the wildfire organism. These 3 strains were transferred at weekly and monthly intervals on potato-dextrose agar for a period of  $1\frac{1}{2}$  years. About 30 tubes of the untransferred original culture of each strain were kept in a refrigerator at 8° C. together with the weekly and monthly transfers.

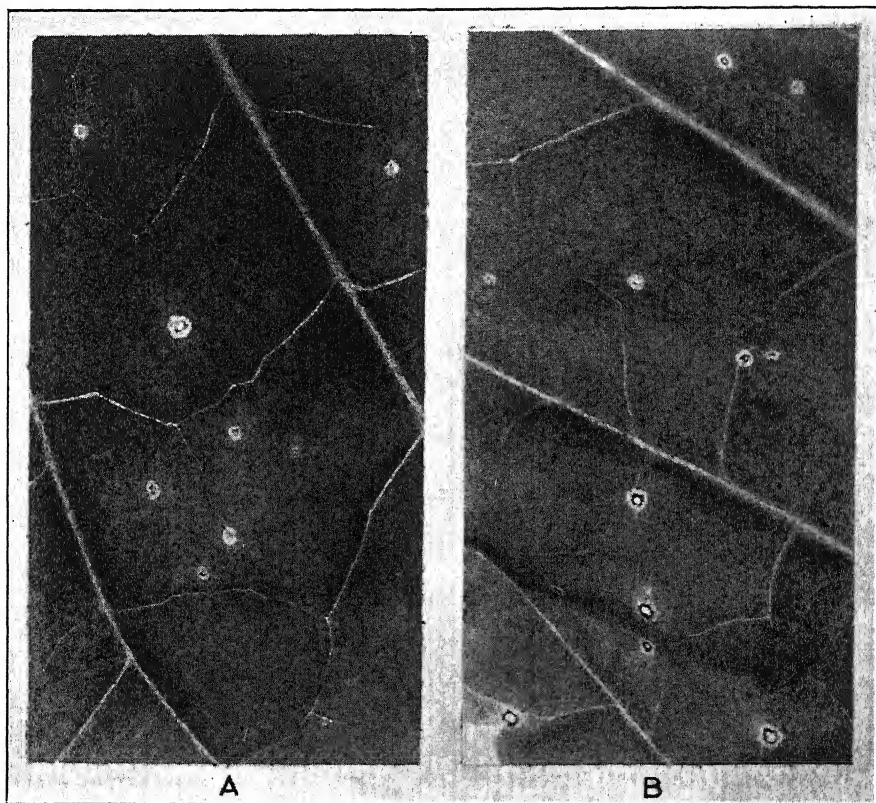


FIG. 3. Slightly enlarged lesions of *Bacterium angulatum* and a non-toxin producing culture of *Bact. tabacum*, by spray inoculation. A. Lesions produced by *Bacterium angulatum*. B. Lesions produced by a non-toxigenic culture of *Bacterium tabacum*. Note marked similarity to A.

The inoculum was prepared by first seeding all the cultures to be tested in bouillon for a 24-hour period to insure viability of the organisms. Transplants from broth to potato-dextrose agar slants were made. After an incubation period of 16 hours at room temperature, each agar slant was washed off with sterile tap water. This method eliminated much of the toxin which may have accumulated in the culture prior to the test. Needle-puncture

inoculations with the aqueous suspensions of the several cultures were made to tobacco plants in order to determine the relative toxin-producing ability of the various subcultures of *Bacterium tabacum*. Readings were taken after a period of 2 weeks and the diameter of the halo, measured in millimeters, was the criterion used in determining the amount of toxin produced. There was found to be a gradual decrease in toxin production in all cultures during the 18-month period over which the experiment was conducted. The results often were complicated by the fact that the toxin-producing ability of the component bacteria of a culture proved extremely heterogeneous. A culture, apparently highly virulent by virtue of the toxin production of a fraction of the constituent organisms, was often found to consist largely of cells that had lost their toxin-producing ability. The greatest percentage of nontoxigenic organisms after 18 months of subculture was found present in the weekly transfers with a lesser percentage in the monthly and original transfers. These conclusions were reached after a random selection and inoculation to tobacco plants of about 50 colonies of each culture. Two stages in the attenuation of the toxicity factor are illustrated in figure 1. It should be noted that these results are not in general agreement with those secured earlier in this laboratory (7). The difference may be accounted for by the fact that the experimental approach in the earlier investigation differed somewhat from that employed in this work.

By plating out on potato-dextrose agar, it generally was found that the number of organisms present in the lesions that showed no halo development was as great as was the number of organisms in the lesions in which there was a maximum halo development. It, therefore, was concluded that the nontoxigenic cultures of *Bacterium tabacum* possessed the ability to live and multiply in the host tissue, and, as far as could be judged from infection trials, apparently differed from the more commonly described wildfire type only in the inability to secrete exotoxin.

Nontoxin-producing wildfire organisms were isolated readily from all 3 strains after they had been carried *in vitro* for 18 months. The cultures were plated out on potato-dextrose agar, and individual colonies were picked and seeded in bouillon. Inoculations were made to tobacco plants with the various isolates in order to determine their toxin-producing ability. By the use of the above method, about 30 nontoxigenic cultures from the 3 strains were obtained and used for further study. It should be recalled that these strains originated from single-cell cultures of *Bacterium tabacum* carried continuously in culture, with no opportunity for contamination with cultures of *Bact. angulatum*.

When aqueous suspensions of the nontoxigenic wildfire organisms were inoculated to tobacco plants by means of an atomizer, so as to facilitate stomatal infection, it was found that brown necrotic spots were produced that could not be distinguished from angular-leaf-spot lesions (Figs. 2 and 3).



Certain of the 30 nontoxigenic isolates were found to be extremely aggressive and to cause almost complete breakdown of the susceptible leaf tissue when atomized on the plant (Fig. 4). The majority of organisms, however, were only moderately destructive and produced small brown necrotic spots, more typical of angular leaf spot. Simultaneous inoculation to individual tobacco plants with *Bacterium angulatum* and the nontoxigenic cultures of *Bact. tabacum* demonstrated that the course of these diseases in the host plant is identical. Within 18 to 24 hours after inoculation, small, translucent, water-soaked spots appeared that later turned brown and necrotic. In most cases the lesions were confined to small areas about  $\frac{1}{8}$  in. in diameter. A chlorotic halo was never found present when nontoxigenic wildfire cultures or angular-leaf-spot bacteria were used as the source of inoculum.

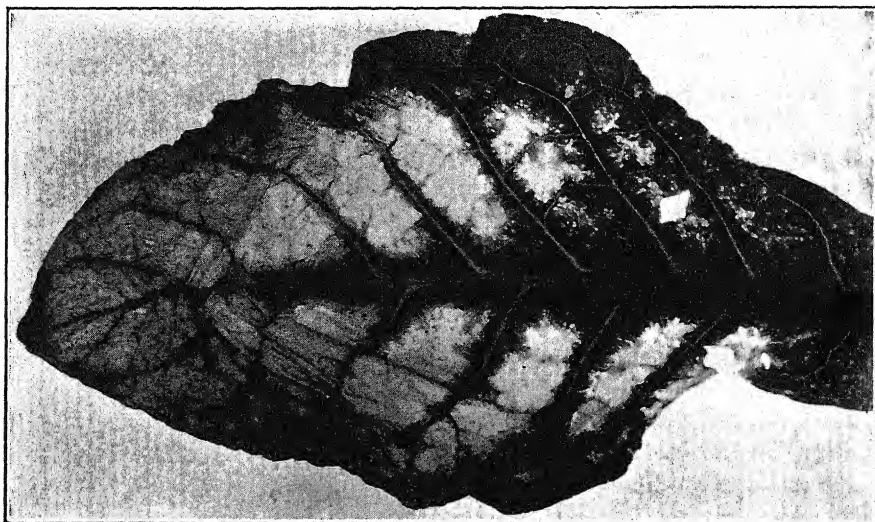


FIG. 4. Tobacco leaf inoculated with an extremely aggressive nontoxigenic culture of *Bacterium tabacum*. Infection produced by spray inoculation and without water soaking of tissue.

This toxigenic attenuation appears not to be associated with any detectable cultural change in the organism, and apparently is not correlated with dissociation of the bacteria into smooth and rough variants. The nontoxin-producing cultures of *Bacterium tabacum* appear to be fairly stable, and all attempts to restore the toxicity factor by carrying these organisms in a variety of ways on several different media have failed. There has been no indication toward reversion to the toxin-producing type of wildfire organism.

#### DISCUSSION

It is obvious from the results of this investigation that a comparison of *Bacterium tabacum* and *Bact. angulatum*, *in vitro*, does not permit definite



identification or separation of one from the other. The earlier distinguishing features suggested by Fromme and Murray (4) and by Wolf (17) could not be corroborated. On the other hand, serological studies on single-cell strains of wildfire and angular-leaf-spot organisms that possessed known pathogenicity indicate that they are indistinguishable by these methods. These results are in accord with the conclusions reached by Stapp (13), and, in general, there appeared at first to be good reasons for considering the organisms identical. It is not proposed to enter into a discussion of the significance of serological and related tests, as a means of determining identity or relationships in this connection. Manifestly, definitions and interpretations are particularly involved.

While there is admittedly much room for a reexamination of species distinctions, and the elimination of synonymous names, the present tendency in plant pathology is to take due cognizance of inheritable variations within the species concept, and to recognize that such variants or varieties may be more significant pathologically than are certain other diagnostic features within a related group. It is, therefore, difficult for those who have studied intensively wildfire and angular leaf spot, in the laboratory, or who have observed their occurrence, symptomatology, and general behavior under field conditions, to accept the hypothesis that they are caused by the same organism.

Nevertheless, we are faced with the additional rather conclusive demonstration that when *Bacterium tabacum* loses its toxin-producing power (which it may apparently readily do in culture, and which is quite likely under natural conditions), we have an organism that may produce symptoms indistinguishable from those produced by *Bact. angulatum*. Symptoms again are not, within certain limits, to be regarded as a valuable diagnostic criterion, nor can it be safely said that any criterion now available is strictly reliable in an attempt to establish such identities.

This comparative study leads the writer to conclude that although it may be acceptable to regard *Bacterium angulatum* as a variety of *Bact. tabacum*, the evidence is not yet sufficient, nor is it technically desirable to combine them under one species name, namely *Pseudomonas tabaci*, as suggested by Stapp (13).

Some problems of practical interest are suggested by the results. The first of these relates to the probable origin of either disease following the occurrence of the other. Fortunately, it seems most improbable that the wildfire organism may originate from mutations of the angular-leaf-spot organism. Despite the apparent "spontaneous" occurrence of the former in some localities and fields, the infrequency of such associations in the almost universal presence of angular-leaf spot and the failure to find a suggestion of such a transformation under experimental conditions argue against such a theory.

Although the wildfire organisms may very likely "degenerate" to a form similar to the angular-leaf-spot organism, it is difficult to estimate the significance of such nontoxigenic wildfire bacteria under field conditions. It might be argued that *Bacterium angulatum* is nothing more than a nontoxin-producing variant of *Bact. tabacum*, since it has been shown that these 2 bacterial types cannot be differentiated by the various diagnostic tests used. This suggestion, however, is admittedly speculation, and until it is demonstrated by field experimentation that such a change can be brought about under natural conditions, the supposition will remain merely an interesting hypothesis.

Control experiments with angular leaf spot are greatly hampered by the small, indefinite lesions often formed, particularly in the seedbeds when the amount of infection is low. It is sometimes very important to be certain whether or not a single lesion exists in such trials, and this is often very difficult to do with the use of the angular-leaf-spot organism. In the instance of wildfire, however, the presence or absence of the disease is much more quickly and certainly established. In view of the close relationship of the two organisms, it is very likely that the best control methods for both diseases will eventually be identical, as they essentially are at present. Experimental work actually aimed at the control of angular leaf spot may perhaps be more easily and certainly carried out by using the wildfire organism in place of *Bacterium angulatum*. Such differences as may possibly exist in the efficiency of and response to control measures may, perhaps, be readily established following the more reliable work made possible with the wildfire organism.

#### SUMMARY

The problem under consideration is concerned chiefly with the possible relationship or identity of two bacterial pathogens of tobacco, namely *Bacterium tabacum* Wolf and Foster, and *Bact. angulatum* Fromme and Murray.

The progeny of 6 single-cell strains of *Bacterium angulatum* and 4 single-cell strains of *Bact. tabacum* of known pathogenicity were used in this study.

Attempts at securing differential characters of the above organisms by means of morphological, cultural, physiological, and serological methods have failed. The individual strains of each organism used yielded greater differences than did the organisms of the two bacterial types. These strain variations might explain certain of the discrepancies found to exist in the earlier reports.

*Bacterium tabacum* was shown to have a tendency to lose its ability to secrete a soluble exotoxin when carried in culture for prolonged periods of time with or without repeated transfer. In some instances, the aggressiveness or invasive power of the nontoxin-producing cultures of *Bact. tabacum*

was not found to be impaired; more often it proved to be approximately that of *Bact. angulatum*.

The symptoms produced by the nontoxigenic wildfire bacteria were found to be indistinguishable from those produced by *Bacterium angulatum*, the causal organism of angular leaf spot of tobacco.

It is believed that, while it may be acceptable, because of the close relationship of these organisms, to regard *Bacterium angulatum* as a variety of *Bact. tabacum*, it does not seem desirable, with the evidence available, to combine the two organisms under a single species name as is done by Stapp.

In view of the close relationship that exists between *Bacterium tabacum* and *Bact. angulatum*, it is suggested that studies relating to the control of both diseases might be carried out more easily and certainly with the use of the wildfire organism.

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# A LEAF AND TWIG DISEASE OF HEMLOCK CAUSED BY A NEW SPECIES OF ROSELLINIA

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## INTRODUCTION

In 1914 Graves<sup>1</sup> reported finding a leaf and twig disease associated with a species of *Rosellinia* on 3 hemlocks, *Tsuga canadensis* (L.) Carr., in western North Carolina. He described the death of leaves and twigs and the investment of the affected small branches, leaf petioles, and leaf bases by a growth of grayish or yellowish-brown mycelium that produced small, more or less globular to dome-shape fruiting bodies, where the mycelium was well developed. During the summer of 1935 apparently the same disease was found by E. E. Ripper, of the U. S. Forest Service in the Pisgah Division of the Pisgah National Forest, Transylvania County, N. C., where 2 of the 3 trees that Graves reported were located. A large number of diseased trees subsequently were found.

The hemlock throughout a large part of the southern Appalachian region is confined to stream banks in coves, and the disease occurred in abundance only in such locations. In the vicinity of John Rock, near the town of Pisgah Forest, it was common in coves having both northern and southern exposures. A great many coves from 15 to 40 miles north of John Rock and west from Asheville, N. C., to the Tennessee line along the French Broad River, were unsuccessfully scouted for the disease.

## CHARACTERISTICS OF THE DISEASE

The disease was studied intensively in East Horse Cove. Nearly every hemlock, both large and small, within 10 yards of the creek, for the distance observed (about a mile), showed some infection. Although the extent of development of the fungus varied considerably on different trees, those nearest the creek usually were infected most abundantly. In most cases hemlock growing on flats at some distance from the creek at the lower end of the cove did not appear to be affected, and where the organism was noted on such trees the attack was slight. The fungus appeared to reach its most prolific development, and the most leaves and twigs were killed, on those parts of branches nearest the trunk and farthest down the stem. Infection appeared to decrease from the base upward and from the trunk outward. Very few of the outermost twigs appeared to be infected. The maximum height at which infection was noticed was about 30 feet. A rough estimate

<sup>1</sup> Graves, A. H. Notes on diseases of trees in the Southern Appalachians III. *Phytopath.* 4: 63-72. 1914.

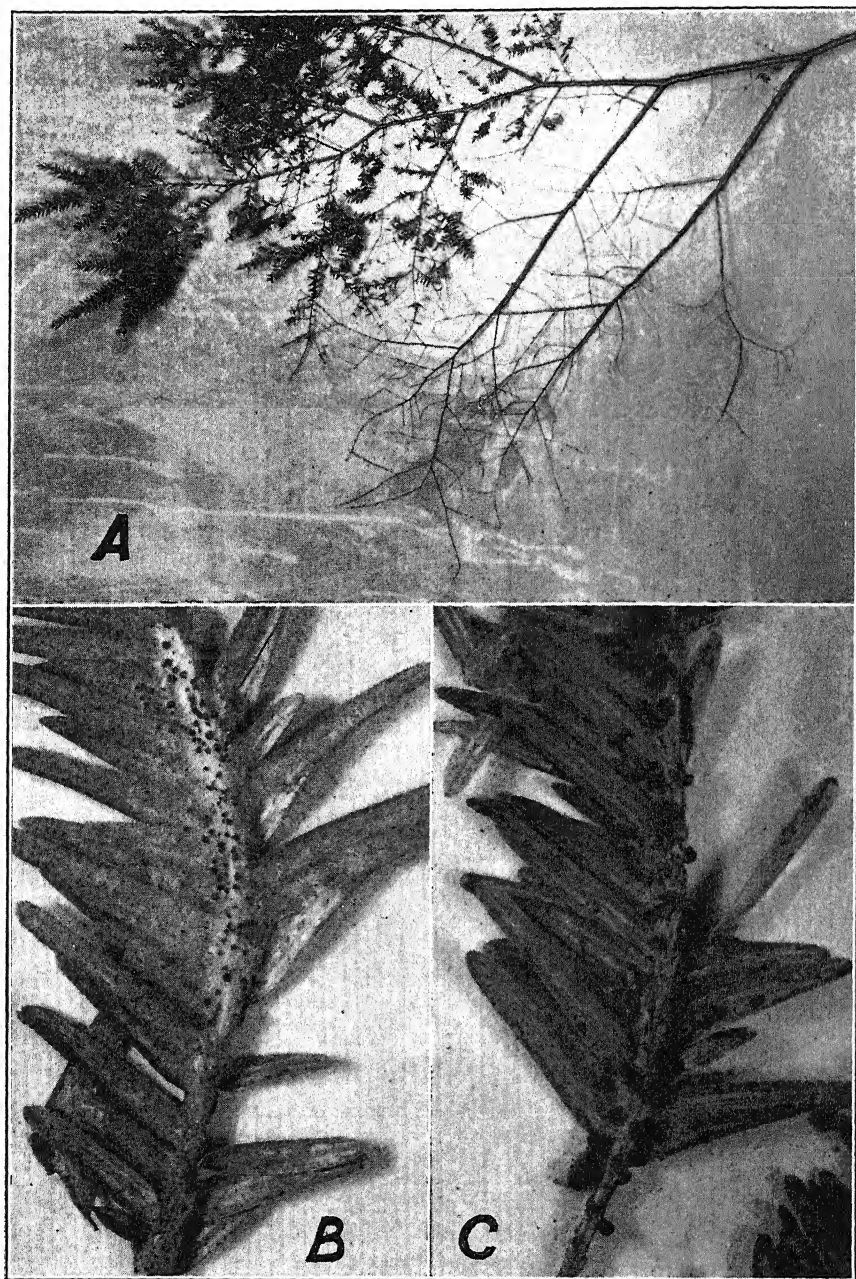


FIG. 1. A. A diseased branch, showing living, dying, and killed twigs. B. Under side of infected twig, showing abundant vegetative growth of *Rosellinia herpotrichioides* and immature perithecia. C. Twig similar to B, showing shrunk superficial thallus and mature perithecia.

of the reduction in foliage due to the fungus along the creek in this cove indicated that of the trees under about 15 feet in height about 20 per cent had lost from 50 to 80 per cent of their foliage, 30 per cent had lost 30 to 50 per cent, and the remaining 50 per cent up to 30 per cent. No tree was found to have been killed by the disease. Killed leaves still on the tree were included in the estimate of foliage lost. A branch partly defoliated as a result of the disease is shown in figure 1, A. The facts that the disease appeared most severe on the inside and lower parts of trees, nearest water, and in the most shaded places indicate that moisture favors it.

#### DESCRIPTION OF THE DISEASE

The general appearance of the vegetative part of the fungus and its effects on the host are somewhat similar to the leaf and twig diseases of western conifers caused by *Herpotrichia nigra* Hartig and *Neopeckia coulteri* (Peck) Sacc.<sup>2</sup> The needle-bearing parts of twigs become densely covered with a grayish-brown mycelial mat. While this mat is often so thick on the under side of a twig as to cover completely the twig and appended leaves on that side (Fig. 1, B), on the upper side there is usually almost no surface mycelium visible to the naked eye. However, under the microscope, surface mycelium can be found on both sides. The mycelial mat varies from felty to cobwebby or mold-like in appearance on the under surface. Small stems may be completely covered with a mantle of mycelium for several inches and yet the bark beneath the mantle may remain green. Considerable green foliage may be found beyond such a mantle. However, leaves attached to that part of a stem covered with a mantle were dead. The leaves apparently do not remain green long after their bases are invested. The perithecia are produced in abundance in the dense mycelial mats on the lower surfaces of twigs and leaf bases. After the leaves have died the mycelium loses its mold-like appearance and becomes flattened against the twigs and leaves, leaving the perithecia appearing as though they had been glued to the surfaces of the twigs (Fig. 1, C).

Although the pathogenicity of this fungus has not been established by inoculations there can be little doubt that it is responsible for the death of the leaves and twigs covered by its mycelium. Whether it achieves the death of affected parts through a parasitic relationship with the host or through the smothering effect of an epiphytic mycelium has not been established.

#### TECHNICAL DESCRIPTION OF THE CAUSAL FUNGUS

##### *Rosellinia herpotrichioides*, n. sp.

Mycelial mats (subiculum), dense, light gray, forming on under side of leaves and branches, flattening out and becoming less conspicuous as the

<sup>2</sup> Sturgis, W. C. *Herpotrichia* and *Neopeckia* on Conifers. *Phytopath.* 3: 152-158. 1913.

perithecia mature; perithecia appearing as small black bodies imbedded in the subiculum, with only the bases invested at maturity, black, carbonaceous, wrinkled; ostioles distinctly papillate, gregarious (sometimes coalescing), globose, .5 to .9 mm. in diameter; asci short stipitate, cylindric, with thickened gelatinous pore at apex,  $185-210 \times 11-14 \mu$ , 8-spore; ascospores diagonally 1-seriate, dark brown, unicellular, ovate-oblong, inequilateral, with small groove on one side, sometimes slightly apiculate,  $23-26 \times 9-10 \mu$ ; conidia closely associated with the mycelium on the dorsal side of leaves, ovoid hyaline,  $5-8 \times 3-5 \mu$  in diameter, borne on Botrytis-like conidiophores.

Subiculum effusum, griseum; peritheciis e subiculo emergentibus, gregariis, globosis, nigris, carbonaceis, rugosis, ostiolo papilliformi, .5-.9 mill. diam., ascis breve stipitatis, cylindricis,  $185-210 \times 11-14 \mu$ , octosporis; sporidiis monostichis, ovate-cymbiformibus, inaequilateralibus, atrofusces, interdum leniter apiculatis,  $23-26 \times 9-10 \mu$ ; conidiis consociatis ovoideis, hyalinis,  $5-8 \times 3-5 \mu$  diam.

Hab. in foliis et ramis viventibus *Tsugae canadensis*. Mt. Pisgah, North Carolina (70927 type, 70926 and 70990 co-types).

A type specimen has been deposited in the mycological collections of the Bureau of Plant Industry.

#### COMPARISON OF *R. HERPOTRICHIOIDES* WITH RELATED SPECIES

It has not been possible to find a described species of *Rosellinia* identical with this one. It is similar to *R. subiculata* Schw. in its habit of forming perithecia on a conspicuous subiculum, but the spores of that species are only one-half as large, and the substratum on which they occur is entirely different. In fact, the habit of growing over living leaves and branches at a considerable height from the ground separates this fungus from any other species of this group.

*Rosellinia quercina* Hartig<sup>3</sup> has been described as a parasite, but its habit of forming mycelial strands and its occurrence on roots of deciduous trees indicate that it is not closely related to the species herein described. These differences also hold true for *R. arcuata* Petch and *R. bunodes* (B. & Br) Sacc., as described by Petch,<sup>4</sup> and, furthermore, their spores are significantly different. *Hypoxyton pruinaum* (Klat.) Cke., although parasitic,<sup>5</sup> causes an entirely different type of injury to the host and is structurally distinct.

*Rosellinia thelena* (Fr.) Rab. frequently grows over dead coniferous twigs and leaves, but it has a much darker subiculum, hyaline appendages on the spores, and is not known to be pathogenic.

<sup>3</sup> Hartig, R. Der Eichenwurzeltödter, *Rosellinia quercina* M. Untersuch. Forstbot. Inst. München I: 1-32. 1880.

<sup>4</sup> Petch, T. Diseases of the tea bush. 331 pp. Macmillan and Co. London and New York. 1923.

<sup>5</sup> Povah, A. Hypoxyton poplar canker. Phytopath. 14: 140-145. 1924.



The fungus that seems to be more nearly comparable microscopically and from the standpoint of host relationship is *Hypoxylon diathrauston* Rehm. This species has been collected several times on dead twigs of *Abies* in Colorado and on twigs of *Tsuga mertensiana* (Bong.) Sarg. in Oregon. The perithecia and spores are slightly larger and no subiculum has been seen on any of the specimens examined. The perithecia of *H. diathrauston* occur more frequently in coalescent groups than do those of the newly described species.

It was observed that the apex of the asci of *Rosellinia herpotrichioides* contains an enlarged gelatinous plug or pore. Figure 2, A and E, shows

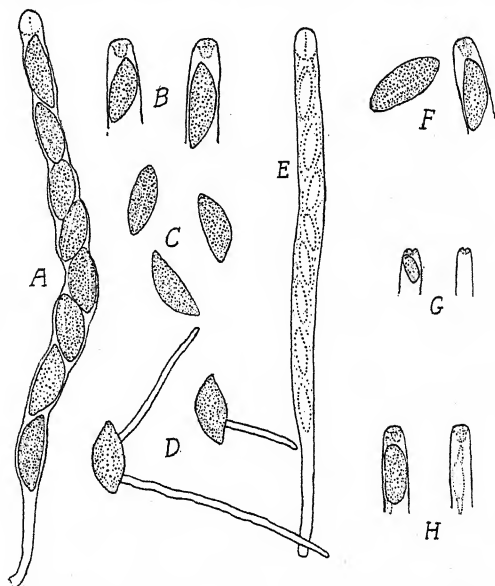


FIG. 2. A-E. *Rosellinia herpotrichioides*. A. Mature ascus. B. Ascus tips showing pores. C. Mature ascospores. D. Germinating ascospores. E. Immature ascus. F. Ascospores and ascus pore of *Hypoxylon diathrauston*. G. Ascus tips of *R. subiculata*. H. Tips of mature and immature asci of *R. thelena*.

these plugs when mounted in an alcohol and glycerine solution. Figure 2, B, illustrates their appearance when mounted in KOH solution. An examination of *R. thelena* and *H. diathrauston*, Figure 2, H and F, disclosed these species to have a very similar type of gelatinous plug. *R. subiculata* has only a very slight thickening around the pore (Fig. 2, G). Hartig<sup>6</sup> also illustrated a very similar type of ascus pore for *R. quercina*.

The shape of the ascospores of the new species and the groove shown on one side of the spores are similar to those of *H. diathrauston* (Fig. 2, C and F).

<sup>6</sup> See footnote 3.

Ascospores of *Rosellinia herpotrichioides* sown on Difco cornmeal agar germinated in 18 to 24 hours at a room temperature of about 75° F., with the production of one or two germ tubes from the grooves in the sides of the spores (Fig. 2, D). With continued growth a loose, light gray hyphal mat was formed over the substratum. No conidia were formed in these cultures and no attempt was made to induce their formation by use of special media.

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## PHYTOPATHOLOGICAL NOTES

*Primary Infection of Setaria italica* (L.) Beauv. by *Sclerospora graminicola* (Sacc.) Schroeter.—The rôle of the oospores of *Sclerospora graminicola* (Sacc.) Schroeter had long been assumed, but it was not until 1925 that definite experimental data relating to their function were obtained by Melhus and Van Haltern.<sup>1</sup> While this investigation left no doubt as to the pathogenicity of the oospores, the experiments were conducted without the investigators' having actually seen the germination of the oospores, and it was not until the work of Hiura<sup>2</sup> that infection was shown to take place through

<sup>1</sup> Melhus, I. E., and F. Van Haltern. *Sclerospora* on corn in America. *Phytopath.* 15: 720-721. 1925.

<sup>2</sup> Hiura, M. Mycological and pathological studies on the downy mildew of Italian millet. *Jour. Faculty Agr. Hokkaido Imp. Univ.* 36: 121-283. 1935.

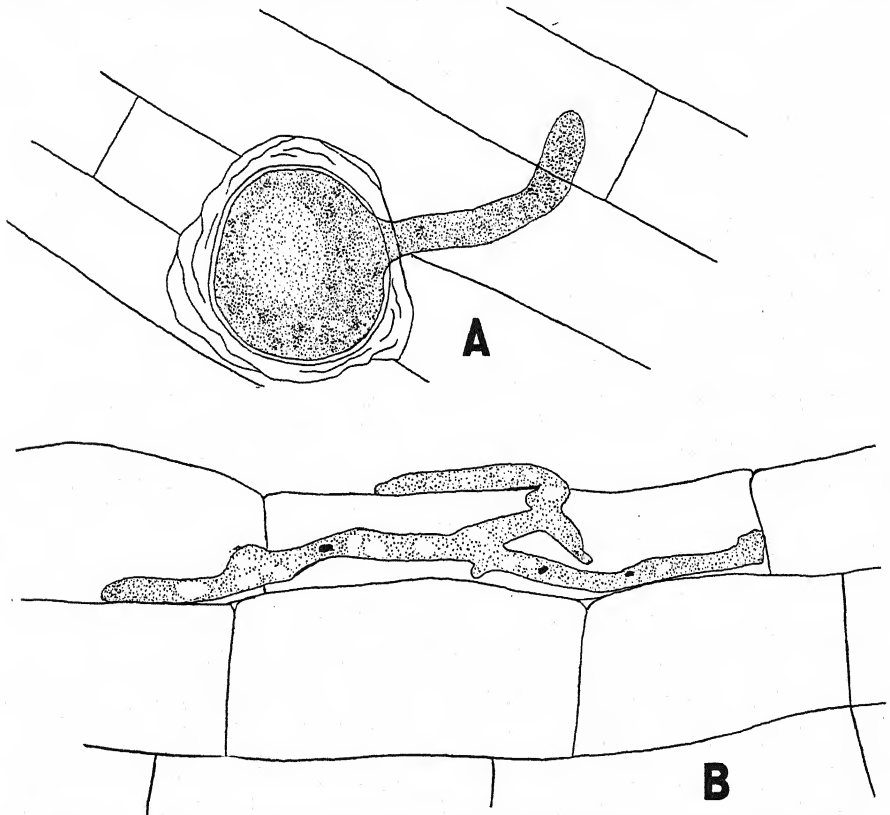


FIG. 1.  $\times 400$ . A. A drawing of an oospore which had germinated. The germ tube was seen to have penetrated into the epidermis of the root of a *Setaria italica* seedling 24 hours old. B. A drawing of a portion of the epidermis and cortex from a sectioned *Setaria italica* root. The infection hypha had branched within the epidermal cell.

the root, mesocotyl, or coleoptile of the young seedlings of *Setaria italica*. The present investigation, of which this is a preliminary note, was undertaken to show the method by which the oospores infect the host and the subsequent development of the pathogene in the plant.

Seeds of the Siberian variety of *Setaria italica* were covered with oospores collected from standing *Setaria viridis* plants and sown on moist cotton in Petri dishes, the covers of which had also been lined with moist cotton. At 24-hour intervals after the seeds commenced to germinate, seedlings were cut from the fruits, fixed, embedded in paraffin, sectioned, and stained. Temporary mounts of whole seedlings also were made with the acetocarmine method.

Under the conditions of these experiments it was found that the germ tube (Fig. 1, A), or a branch of it, entered directly into the epidermal cells of the root, coleorhiza, mesocotyl, or coleoptile. Most frequently the infection took place in the young root or coleorhiza. In most cases the infection hypha was observed to have grown in contact with the outer wall of the epidermal cell before having penetrated into the cell (Fig. 1, B). For a time the further growth of the fungus was intracellular, but subsequently the pathogen became intercellular. From the root or the coleorhiza the mycelium grew into the cotyledonary (scutellary) node and from this into the first internode. Once having gained entrance to the stem the growth of the fungus was directed almost entirely toward the embryonic region of this organ. The path taken by the mycelium was either in the cortex or in the stele.



FIG. 2.  $\times 450$ . A photomicrograph of the growing tip of an infected stem of *Setaria italica* that had been infected with *Sclerospora graminicola*. The fungus has entered the young leaves.

For some time during the growth of the seedling the mycelium was seen directly back of and penetrating into the embryonic region of the stem (Fig. 2). From this region the fungus spread to the young leaves and branches.

The method used to infect the seedlings of *Setaria italica* with *Sclerospora graminicola* seemed to simulate conditions existing in the soil. In the fall the oospores, as well as the seeds, fall to the ground and become mixed together. In the spring the oospores germinate and infect the young seedlings before they emerge from the ground as has been shown by Melhus, Van Haltern and Bliss.<sup>3</sup> Since the coleorhiza is normally the first part of the seedling to emerge from the seed and, since its growth is rather limited, it would seem to be the part most accessible for the attack of the fungus. The results of this investigation indicate that the primary root and the coleorhiza are the parts of the seedling most frequently affording entrance for the fungus.—E. S. McDONOUGH, Iowa State College, Botany Department, Ames, Iowa.

<sup>3</sup> Melhus, I. E., F. Van Haltern, and D. E. Bliss. A study of *Sclerospora graminicola* (Sacc.) Schroet. on *Setaria viridis* (L.) Beauv. and *Zea mays* L. Iowa Agr. Expt. Sta. Res. Bull. 111: 297-338. 1928.

*The Degree of Bunt Resistance Necessary in a Commercial Wheat.*—The production of wheat varieties resistant to bunt (*Tilletia tritici* and *T. levis*) is engaging the attention of a number of plant breeders at the present time. Briggs<sup>1</sup> has shown that, even in a cross between a variety like Martin, completely resistant to the physiologic race of *T. tritici* used in this study, and susceptible varieties, some of the resistant hybrids do not show the high resistance of Martin. In a broader view this incomplete resistance is true also of a number of the best bunt-resistant varieties, as many of them are slightly susceptible to some races of the fungus. Published data<sup>2</sup> indicate that this slight susceptibility is due to the presence of modifying factors. This is of importance where the backcross method of breeding is being used, because that method will incorporate in the hybrid any modifying factors carried by the recurrent parent. The question naturally arises as to the suitability of such slightly susceptible lines for commercial production from the standpoint of bunt resistance. Will enough inoculum be produced to maintain the disease under the usual method of handling?

In 1934, two slightly susceptible strains of wheat were chosen with the view of investigating this point. The first was from Martin × White Federation<sup>3</sup> and the other from Martin × Sonora.<sup>4</sup> (The superscripts 3 and 4 indicate the number of times the recurrent parent has been used in the

<sup>1</sup> Briggs, F. N. Inheritance of resistance to bunt, *Tilletia tritici* (Bjerk.) Wint., in wheat. Jour. Agr. Research 32: 973-990, illus. 1926.

<sup>2</sup> Briggs, F. N. Factors which modify the resistance of wheat to bunt, *Tilletia tritici*. Hilgardia 4: 175-184. 1929.

cross). The latter was chosen because it contained the highest amount of bunt found in the resistant lines. The former represented the other extreme except for completely resistant strains, which were still present in each cross. White Federation and Sonora, the commercial parents, were used as check varieties. Fiftieth-acre plots of each were sown with seed that carried the maximum load of bunt spores that would adhere to them. The inoculum was from the same collection of bunt that has been used in all the studies at the University Farm, Davis, Calif. It has been designated by Reed<sup>3</sup> as physiologic race III of *T. tritici*.

The hybrid strains and varieties were threshed and the seed planted for 2 successive years without adding any further inoculum. The bunt percentages recorded in table 1 are the results of counts of 1,000 or more heads in each case.

TABLE 1.—*The percentages of bunt in hybrids and varieties of wheat from inoculated seed for the 1934 crop*

Variety or hybrid selection	Total of bunted heads		
	1934	1935	1936
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Martin × White Federation <sup>3</sup> .....	0.8	0.0	0.0
White Federation .....	51.8	63.7	65.9
Martin × Sonora <sup>4</sup> .....	5.6	0.4	0.0
Sonora .....	78.1	71.6	77.2

It will be seen that the 0.8 per cent of bunt in the White Federation hybrid did not produce enough inoculum to cause any infection in 1935. An examination of the entire plot in 1935 and in 1936 failed to reveal a single bunted head. Starting with 5.6 per cent of bunt in the Sonora hybrid in 1934, the percentage dropped to 0.4 in 1935. No bunt was found in the entire plot in 1936. An examination of both parent varieties shows that conditions were favorable for bunt infection during the 3-year period.

The above results indicate that varieties of wheat slightly susceptible to the race of bunt used will be suitable for commercial production even under conditions that are very favorable for the disease.—G. A. WIEBE, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and FRED N. BRIGGS, Division of Agronomy, University of California, Davis, Calif.

<sup>3</sup> Reed, G. M. Physiologic races of bunt of wheat. *Amer. Jour. Bot.* 15: 157–70. 1928.

# SOME MEASUREMENTS OF DETRIMENTAL EFFECTS OF THE ROOT-KNOT NEMATODE ON THE PINEAPPLE PLANT<sup>1</sup>

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Aside from the influence on yield of soil infestations with the root-knot nematode, *Heterodera marioni* (Cornu) Goodey, as deduced from control experiments of various kinds, very little has been published on the measurable effects of the nematode on infested plants. Godfrey (4) in reporting on soil fumigation experiments for nematode control, gives considerable data on the effects of various soil treatments upon different features of pineapple-plant growth. Collins and Hagan (1, 8, 9) report upon the effects of heavy soil infestations upon pineapple root and top growth in soil boxes and tubs of limited capacity, in connection with varietal-susceptibility studies. Magistad and Oliveira (10) report the effects of heavy infestations on nitrogen absorption in plants, and on plant growth. Reference also is made herein to a previous paper (6) on the relations of the nematode to the pineapple plant. It is the object of this paper to report studies on the relation between extent of nematode infestation and the several morphological and physiological features of plant growth, supposedly influenced to some extent by the nematodes, under field conditions.

## EXPERIMENTAL WORK

### Maui Area

In the spring of 1931 a generally healthy pineapple field on the island of Maui, Hawaiian Islands, with the plants about 8 months in the ground, was observed with a more or less sharply delimited spot of about  $\frac{1}{3}$  acre area in which the plants were distinctly dwarfed and off color. Examination of a few plants from the failing area disclosed abundant root knot, while surrounding areas seemed relatively free from it. As the complete range, from extreme nematode infestation to its complete absence obtained in a limited area of apparently similar soil, the opportunity seemed to favor a study of the quantitative relationships between the degree of infestation of the plants and various measurable details of plant growth.

It seemed desirable to determine whether or not some factor other than nematodes might have been responsible for at least some of the difference in plant growth between the failing area and the adjoining healthy portions

<sup>1</sup> Acknowledgement is made of credit due Juliette M. Oliveira for careful laboratory measurements and counts. Raw data are eliminated from this paper because of the very great amount of space they would occupy.

of the field. M. B. Linford, of the Pineapple Experiment Station, kindly consented to make a study of soil samples from the two areas to determine the presence or absence of the known root-disease fungi of the pineapple. His report on the "root-bait" method of determination showed findings on root-rotting pythiums and phytophthoras in different soil samples as follows:

Healthy area, positive 7, negative 5; border area, positive 13, negative 7; failing area, positive 13, negative 17, together with the comment "Comparison of the records of these tests with the notes on the sources of samples suggests no direct relationship between presence of root rotting fungi and weakness of the plants. The natural interpretation is, therefore, that some other factor was the cause of plant failure. It is evident, however, that many weeks had intervened between the beginning of the actual injury and the collection of these soil samples, and consequently that the results of these tests are not actual proof that the fungus here considered was unimportant." No signs of the typical injury occasionally produced in pineapple by the garden symphylid, *Scutigerella immaculata* Newport, or of any other root-attacking arthropod was found particularly abundant in the failing area. There were no prominent symptoms of disease or insect attack in the aerial parts of the plants. No particular difference in soil type was evident. Fertilizer practices were uniform throughout the entire field. It would seem that, in all probability, the obvious root-knot nematode was the factor principally responsible for injury to plants in the failing area.



FIG. 1. Cowpeas 8 weeks after planting, in soil from the Maui failing area, and a representative plant (extreme right) from the adjoining healthy area. The failing-area plants were all heavily infested by nematodes.

As a still further check on this, soil samples were removed to the Nematology laboratory in Honolulu, placed in root-observation boxes, and planted to nematode-susceptible cowpeas. The plants in the soil from the failing area became heavily infested by nematodes. Their growth was distinctly dwarfed as compared with those grown in the soil taken from the healthy area. (Fig. 1.) Another lot of the same soil was subjected to thorough insolation for 2 or 3 days, with a view to killing the nematodes. Such soil



when planted showed no noticeable difference between the two areas. Still another lot of failing-area soil was given a chloropicrin fumigation according to best procedure (5, 7), to eliminate nematodes as a factor in plant growth. After a liberal reinoculation of the soil with a culture of *Rhizobium*, the resulting plant growth was on a par with that in the nonfumigated healthy-area soil. Thus, the cowpea, as an indicator, gave substantial proof that nematode infestation constituted a major difference between the two areas.

*Methods and Procedure.* Blocks of pineapple plants were selected for study in failing, border, and healthy areas. These plants were removed from the soil, as completely intact as possible, by digging a trench a foot or more away from the plant row and about 15 inches deep, and then carefully picking away from them the soil about the roots until the entire root system was free. The plants were then lifted, the roots washed free from soil, and the plants labelled for further detailed study. Quantitative studies on plant growth were readily made by taking counts, weights, and measurements. Aside from these, the first desideratum was to select a reliable criterion for measuring the extent of nematode infestation. The only possibility appeared to be the actual count of the nematode galls on the excavated root systems. No attempt was made to include the small rootlet galls in the count. Finally, certain physiological conditions challenged attention. Some observers had remarked on the greater amount of red coloration in the leaves of pineapple plants growing in heavy nematode-infestation areas. This red color is due to anthocyanin. Also the question had been raised as to the significance of the dying of leaf tips in nematode areas. Both of these aboveground symptoms were studied quantitatively.

With some of the data taken in the field and some in the Honolulu laboratory, complete plant-by-plant data were recorded under the following designations:

a. Total green plant weight; b. Total number of individual main roots; c. Total root length, obtained by adding the lengths of all individual main roots; d. Percentage of short roots (less than 5 inches); e. Total number of distinguishable main root galls; f. Total number of roots with distinct terminal galls; g. Percentage of roots with distinct terminal galls; h. Number of galls per 100 inches of root length; i. Total length of portions of roots free from galls (estimated by the formula,  $i = c - \frac{e}{2}$ ); j. Total number of leaves down to about 1-inch length; k. Percentage of leaves with dead tips  $\frac{1}{2}$  inch or more in length; l. weight of leaves; m. The percentage, by weight, of the anthocyanin-showing parts of leaves (as excised from the distal ends); n. Fruit yields from selected areas in the failing spot and from the adjoining healthy area. These data were obtained approximately a year after the first studies were made, and, of course, were from different plants.

In analyzing data for both plot averages (Table 1) and correlation determinations (Table 2), step intervals chosen were of such orders as to make for fairly normal variability curves.

*Results.* The data obtained were first analyzed statistically by the two distinct areas, failing and healthy, with border blocks omitted. The healthy population consisted of 75 plants that were almost completely free from nematode infestation (never more than 2 or 3 main root galls on a plant). The failing population, of 105 plants, had an average of  $72 \pm 3.1$  galls per plant of which  $46 \pm 2.0$  were terminal. The results are given in condensed form in table 1.

TABLE 1.—*A comparison of nematode-infested and practically nematode-free areas of field grown pineapples for various features of plant growth*

Character measured	Healthy	Failing	Difference	Per-centage difference
Weight of plant, ounces	89.6 $\pm$ 2.8	74.9 $\pm$ 1.45	- 14.7 $\pm$ 3.15	16.4
Number of roots .....	123 $\pm$ 2.9	158 $\pm$ 3.5	+ 35 $\pm$ 4.5	28.5
Length of roots, inches ...	788 $\pm$ 19	571 $\pm$ 12.5	- 217 $\pm$ 23	27.5
Percentage of roots < 5",	55.7 $\pm$ .98	84.8 $\pm$ .7	+ 29 $\pm$ 1.2	52.5
Number of leaves .....	40.7 $\pm$ .74	40.25 $\pm$ .53	- 0.43 $\pm$ .91	.....
Weight of leaves, ounces	71.8 $\pm$ 2.16	61.1 $\pm$ 1.22	- 10.7 $\pm$ 2.48	15.0
Percentage of leaves with dead tips .....	18.4 $\pm$ .37	19.2 $\pm$ .30	+ 0.8 $\pm$ .45	.....
Percentage of leaves with visible anthocyanin .....	11.7 $\pm$ .35	16.7 $\pm$ .2	+ 5.0 $\pm$ .4	42.8
Yield				
Weight per fruit, lbs.,...	4.15	2.18	- 1.97	47.5
Percentage of No. 1 fruit .....	84	9.0	- 75.0	90

In most cases the differences shown are highly significant. In 2 cases, nematode infestation of the soil appears to have no significant effect. Only where the differences are statistically significant are the percentage differences included in the final column. In the plant-weight data, the difference would have appeared as a greater percentage had it been possible to deduct the weight of the original somewhat irregular planting material from the total final weight. The results in general are sufficiently obvious in the table to require no special discussion.

Another phase of this study consisted in the calculation of correlations between certain variables between which a cause and effect relation might be conceived to exist. Only plants that showed at least some trace of nematode infestation were included. In addition to plants from those blocks

within the border of the failing area, border plants and a few that showed some galls on the roots from the relatively healthy area were incorporated in the calculations. This gave a population of about 140 plants with a reasonably normal variability curve of root-gall counts, upon which all calculations are based.

Garrett (2) was the guide used for all correlation studies. As to the cause-and-effect relationship, one simply has to be guided by his statement, page 253: "The conclusion as to which of two factors is the cause and which the effect is a matter of common sense analysis." The calculated result is, of course, the same in either case.

The simple, 2-variable correlations obtained are included in table 2.

#### Oahu Area

Similar plant-by-plant data taken from a soil-treatment experiment on the island of Oahu, reported elsewhere (4), lend themselves to a similar analysis. Originally this was an experiment to test the efficiency of certain treatments in a soil heavily infested with nematodes, for improving plant growth and yield. For the purpose of this paper it may be considered from the other point of view, *i.e.*, the plots receiving the best treatments and relatively nematode-free at planting time being considered the "normal"; and the untreated and less efficiently treated being considered as having varying degrees of nematode infestation, whose effects are measured in comparison with the normal. In this case the plants were removed from the ground when they were about 5 months old or 3 months younger than those in the Maui experiment. The plot results in relation to treatments given are reported in detail in the previous paper. Disregarding treatments and considering all the plants as a single population, a broad range is obtained in all the variables. The correlations obtained are included in table 2, using the same key as for the Maui data.

#### DISCUSSION AND INTERPRETATION OF RESULTS

A complex statistical situation arises from the interrelationships between the different variables measured. Some of these complexities are discussed, along with the definite results obtained, under the headings of the separate variables measured. The causative variable receives the principal discussion. Total given plant weight.

*b. Total Number of Roots.* The increase in number of roots in the heavily infested areas over the non-infested or lightly infested (Maui data 28.5 per cent, Oahu, 18.7 per cent) appears to be a response to parasitism similar to that occurring with many kinds of parasitism. It may be even temporarily beneficial to plant growth. But the additional burden on the plant of producing new roots at an early stage of growth must inevitably weaken it, for its capacity to produce new roots is limited. If there be an

TABLE 2.—Correlations between different measured variables concerned in the effects of the root-knot nematode on the pineapple plant

Maui data	Weight of plants a	Number of roots b	Length of roots c	Number terminal galls f	Length of roots free from galls i	Number of leaves j	Percent- age dead leaf tips k	Weight of leaves l	Percent- age antho- cyanin portion m
Number of roots									
(b) .....	.347 ± .05	.....	.36 ± .05	.....	.23 ± .054	.55 ± .04	.....	.....	.....
Length of roots									
(c) .....	.578 ± .038	.....	.....	.....	.....	.....	.....	.57 ± .035	.....
Number of galls									
(e) .....	.258 ± .053	.49 ± .043	-.084 ± .055	.96 ± .005	-.213 ± .055	.....	.....	.....	.....
Percentage of ter- minal galls (g) ..	.....	.21 ± .055	-.30 ± .05	.....	.....	.....	.29 ± .05	.....	.43 ± .047
Roots free from terminal galls									
(b-f) .....	.....	.....	.47 ± .042	.....	.....	.....	.....	.....	.....
Galls per 100 in- ches (h) .....	-.12 ± .055	.292 ± .05	-.39 ± .049	.....	.....	.....	.....	.....	.....
Length of roots free from galls									
(i) .....	.60 ± .034	.23 ± .05	.96 ± .005	.....	.....	.....	.....	.....	.....

TABLE 2.—(Continued)

Oahu data	Weight of plants a	Number of roots b	Length of roots c	Yield by plots n
Length of roots (e) .....	.76 $\pm$ .012	.....	.....	.67 $\pm$ .055
Number of galls (e) .....	.....	.....	-.38 $\pm$ .08	.....
Number of roots (b) .....	.....	.....	.61 $\pm$ .018	.....
Percentage terminal galls (g) .....	.....	.11 $\pm$ .027	-.69 $\pm$ .05	.....
Original nematode population .....	.....	.....	.....	-.852 $\pm$ .026

overproduction of new roots early in the growth of the plants, new roots will be failing in the critical period of later growth and fruit production. This is supported by observation, but no data on the point are available.

The positive correlations between number of roots and total length of roots, *bc* Maui data, .36  $\pm$  .05, and *bc* Oahu data, .61  $\pm$  .02, are definite and significant of a contribution of increased number of roots to increased root length.

*c. Total Root Length.* The correlation between root length and plant weight in the nematode-infested population of plants in the Maui area is .57; in the total population, diseased and healthy alike (208 plants), .80; in the Oahu area, .76. Clearly then, other things being equal, the vigor of the plant, in so far as it can be measured by plant weight, is benefited by increase in root length. The same influence is shown by the correlations with weight of leaves, .57, and with yield, .67, (the latter calculation, on Oahu data, being based upon the averages for the 49 plots.)

*d. Percentage of Short Roots.* Maui data only, available.

The Maui data show that there was a significantly greater proportion of short roots in the infested than in the relatively nematode-free area, 85 per cent in the one case and 55 in the other. This might normally be expected to reduce greatly the feeding range of the root system and the plant-food intake. With the pineapple, however, the common practice of fertilizing directly at the plant base may serve to make the abundance of roots in this region something of an advantage, so that the "expected" detrimental effect of short roots is overcome in some part. The correlation between plant weight and percentage of short roots (diagrammed but not calculated), instead of being distinctly negative as might be expected, was not far from 0.

*e, f, g, h, and i,* (relating to gall counts). To follow discussion of other variables.

*j. Total Number of Leaves.* Maui data only.

The correlation of .55 between number of leaves and number of roots (*jb*, Maui data) shows that the two variables are positively and significantly correlated.

*k. Percentage of Leaves with Dead Tips.*

This does not in any way constitute an adequate criterion of nematode damage to the plant. The difference between the infested and the healthy area on Maui, while in favor of the healthy, was not significant.

*l. Weight of Leaves.*

This measurement closely parallels that of the entire plant weight. The leaf weight was reduced about 15 per cent in the infested area. Interestingly, the correlation with root length (*lc*, Maui) was .57, almost identical with that for total plant weight and root length.

*m. Percentage of the Red (Anthocyanin) Colored Portion* of the leaves, by weight.

The Maui data show a statistically significant but scarcely detectable increase in amount of visible anthocyanin in the infested area over the other, 16.7 per cent in the one case, and 11.7 in the other. Possibly, when coupled with other symptoms, such as dwarfing, this may be considered a reliable sign of heavy underground infestation. Its correlation with percentage of roots having terminal galls (*mg*, Maui) is .43, positive, and significant.

*n. Fruit Yields.* Oahu data, only.

The Maui data show a reduction in average weight per fruit in the infested area of nearly 50 per cent. The practical difference is much greater than that, however, for there was a difference of nearly 90 per cent in the proportion of fruits sufficiently large to be classed as "number 1." In the Oahu area the corresponding crop yield difference was about 33 per cent.

With the Maui data it was not possible to calculate correlations of yield with other variables, as the plants from which the other data were taken were, of course, removed from the field and destroyed without bearing fruit. With the Oahu data there was a correlation (*nc*) of .67 between plot yields and plot averages in root length. There was also the high negative correlation of  $-.85$  between plot yields and plot averages in initial nematode population, as determined by indicator plant counts.

The effect on yield of heavy nematode infestation may be considered to be the resultant of the combined effects of such infestation on the growth and physiology of all the parts of the plant, whether or not these effects have been measured.

*e, f, g, h, and i. The Root-gall Relations.*

In these studies the gall counts are the basic measure of extent of nematode invasion of the plants. This appeared to be the only measure possible. It has proved highly satisfactory in indicator-crop studies (3) where primary soil infestation was the principal consideration. With the pineapple plant, however, the situation proved to be vastly more complicated. A reliable "primary infection" or first generation count is impossible because of the great irregularity in the development of pineapple roots and because of the

two distinct types of primary infections (rootlet and main root), and the impossibility of correctly weighting the two to put them on the same basis, since a terminal gall may contain anywhere from 25 to 250 times as many nematodes as a single rootlet gall. Counts made subsequent to the first generation, make for tremendous discrepancies in measurements of magnitude of invasion. By the time plants are 6 or 8 months old several generations of nematodes will have accomplished their detrimental effects on plant growth, often without leaving adequate symptoms to indicate the full magnitude of parasitism involved during the period of their activity. As a measure of extent of infestation, a complete count of the nematodes contained within the roots is not reliable because of the extensive evacuation from old galls. Furthermore, it is too time-consuming to be practicable. However, since main-root galls are clearly distinguishable, and since striking differences occurred between plants in numbers of such galls present, such counts were used in the present study to determine whether or not they could safely be applied as a measure of nematode infestation, and perhaps correlative thereto, of degree of nematode injury to the plant.

The simple 2-variable correlations obtained (Table 2) are significant of the unsatisfactory nature of gall count alone as a measure of the true extent of parasitism of the plants. In the Maui test, with an unquestionably large and important yield difference between failing and healthy areas, with significant differences in plant weight, and in total root length at 8 months of growth, and the correlation between plant weight and root length significant (.573), the negative correlation was only .08 between root length and number of galls per plant. And we have the anomaly of an actual positive correlation of about .26 between plant weight and number of galls (as if increase in number of galls were actually a contributing factor to increased plant growth!). Such a situation certainly required further study.

Consider first the relationship between nematode infestation and number of roots. There is, inherent with the pineapple plant, a tendency toward wide variability in number of roots produced. In the Maui population of 75 healthy plants there was a coefficient of variation of 50 around a mean of 123. For the population of 140 infested plants to show a correlation of .49 between root number and gall count (*be*, Maui data), therefore, indicates some positive relationship between the two variables. Indeed, it is probably mutual in contributory effects. The heavy infestation has brought about an increase in number of roots. The consequent increase in number of root tips has made it possible for more galls to develop, for new infections in main roots occur only in root tips.

In considering the relationship of nematode infestation to total root length per plant, we have to consider a complexity of interrelations. There is

actually a very low nonsignificant negative correlation (*ce* Maui data) of  $-.084$  between root length and total gall count. Considering this in connection with the correlations previously discussed,  $r_{bc} = .36$  and  $r_{be} = .49$  (see table 2), one can calculate the partial correlation  $r_{ce.b}$  (root length on number of galls with number of roots constant) as follows:

$$r_{ce.b} = \frac{r_{ce} - (r_{bc} \cdot r_{be})}{\sqrt{1 - r_{bc}^2} \cdot \sqrt{1 - r_{be}^2}} = \frac{-.084 - (.36 \times .49)}{\sqrt{1 - .1296} \cdot \sqrt{1 - .2401}} = -.31 \pm .052$$

In other words, if it had not been for the increase in number of roots, brought about by the parasitism, the correlation of root length on number of galls would be  $-.31$  instead of  $-.08$ . In the case of the Oahu data, taken considerably earlier in the growth of the plants,  $r_{ce}$  is  $-.38 \pm .08$ , which demonstrates a more direct relationship between number of galls and root length at an early stage than later. The partial correlation was not calculated in this case, but examination of the data shows that it would be very much higher than  $-.38$ .

This complexity of interrelations might be interpreted as meaning that, by increasing the number and, therefore, the total length of its roots, the plant has largely overcome the handicap of infestation. But the final effect of infestation is not to be measured by the length of the root system alone. The correlation of that variable with plant weight is  $.57$  in the Maui test. Some factor, probably unmeasured, has had an additional effect in root length or plant growth, or both. This may be that mentioned under the heading "Number of roots," viz.: the deterrent effect of early heavier root production on the later growth of roots. Indeed, this is definitely indicated by comparison of the two *bc* correlations,  $.61$  at 5 months and  $.36$  at 8 months of plant growth. In the 3 months' difference in time the relatively high early correlation between root length and number of roots has not maintained itself. It is still further indicated by the drop in the *ac* correlation (weight of plants with length of roots) in the same period,  $.76$  to  $.57$ . The two sets of data were taken in different areas and, therefore, are not strictly comparable; but the differences associated with the time of the reading are too striking to be ignored.

Before leaving gall-count data, two other criteria of measurement are to be considered in relation to root length. With the Maui data, the percentage of roots, having terminal galls (*g*, table 2) correlates more closely with the root length variable than does the absolute gall count— $-.30$  as compared with  $-.084$ . With the Oahu data the comparison is  $-.69$  with  $-.38$ . Here, too, the partial correlation  $r_{cg.b}$  (root number being again constant) is significant, it being  $-.55$  in the one case, and  $-.96$  in the other. With the percentage criterion, however, the plant with a heavy root system may be in exactly the same category as the one with a light root system, provided only



that the percentage of roots with terminal galls be the same. Exactly this condition may come about with the "delayed heavy infection" described in a previous paper (6). That it occurs frequently, particularly in the later reading, is shown by the scatter diagrams from which correlations were calculated. The second criterion, studied with the Maui data alone, is the number of galls per 100 inches of root length. Its correlation with the root length variable ( $r_{ch}$ , Maui) is  $-.39$ , the highest of the correlations between root-gall data and root length. Here, too, the correlation with plant weight ( $r_{ah}$ ),  $-.12$  is the expected negative one, instead of the positive  $ae$  correlation, which has seemed inconsistent. The low correlations are explainable in part, as in the case of the percentage criterion, by the undoubtedly frequent occurrence of delayed heavy infection, which placed many cases of heavy root systems in the same category with light roots.

Further attention is now given to plant weight as the most accurate measure of plant growth available. Correlations of .57 and .76, respectively, for the Maui and Oahu data, have been shown to occur between plant weight and root length. These figures are distinctly higher than those correlations in which some form of the gall-count data were compared with the corresponding plant weight data. In other words, the length of root that grows *in spite of nematodes* has greater weight in a positive way in influencing plant growth than has the magnitude of infestation, as measured by any of the root-gall concepts given, in a negative way. Along the same line of thinking, the absolute number of roots that have escaped terminal-gall formation ( $b-f$ , Table 2) shows a correlation of .47 with root length.

To consider partial correlations again, having the simple correlations  $ac = .57$ ,  $ae = .26$ , and  $ce = -.084$ , we can determine the relationship of plant weight ( $a$ ) to root length ( $c$ ) "on its own responsibility," with the seemingly inconsistent variable number of galls ( $e$ ) eliminated in so far as its influence on the others is concerned. Calculating as before, we find that  $r_{ac.e} = .622$ .

With this in mind, when plant weight is correlated with the figures representing healthy or nematode-free portions of root length (estimated by subtracting  $\frac{1}{2}$  inch for each gall for every plant of the population) we, very interestingly, reach an almost identical figure (Maui data)  $r_{ai} = .60$ . With identically the same population of plants that has shown the anomaly of a positive correlation of .258 between weight of plants and number of galls, there is also the very much larger positive correlation of .60 between weight of plants and length of portions of roots free from galls! The concept must be obtained here that, while this is not, directly, a measure of amount of nematode infestation, it is a measure, all other things being equal, of the amount of normally functioning root growth, in spite of nematodes. If one could rely upon the reduction in total root length having been due to nematodes primarily, through their various influences, even though these

influences be not measurable by root-gall count, then the difference between the length of the healthy portion of a root system and an "ideal" root length, as represented by the best of the population of plants, should be a fair measure of nematode damage. This concept admittedly involves some philosophizing, but it is justified in part, at least, by data previously reported (6, p. 419), which shows that every nematode infestation of a magnitude capable of causing a substantial gall shortens the accrued growth during the next 7 days to about 1/6 that of a comparable healthy root.

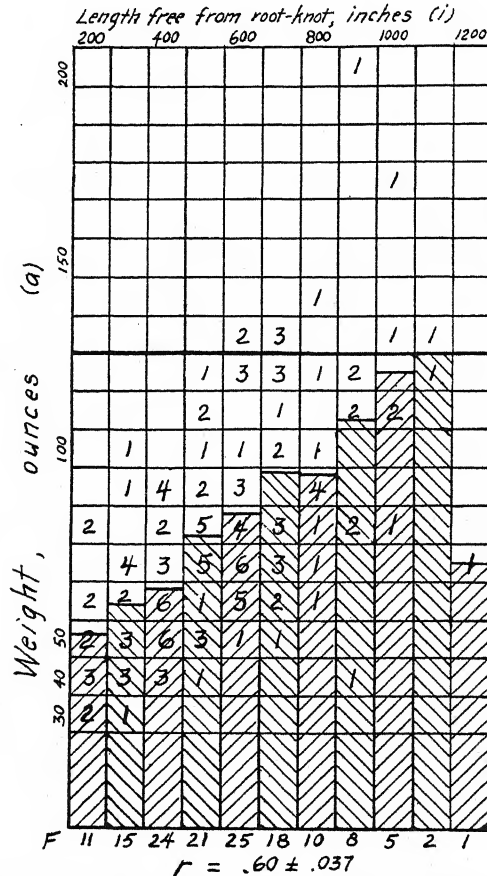


FIG. 2. Correlation surface representing relationship between weight of pineapple plants and length of root systems free from root knot, at 8 months, in the Maui experiment. The shaded columns represent the averages of weights in the 11 root-length categories.

This interpretation of the data is illustrated for correlation *ai* (Fig. 2). The positions of the averages of the columns for weight, corresponding to root length, *i*, class centers, are marked by heavy horizontal lines, and the columns below are lightly shaded. The numbers of plants within each

category are, of course, represented by the appropriate frequency distribution figures. It is to be seen that, except for the last column (represented by a single sample and, therefore, nonsignificant) the distribution of weight averages is remarkably in accord with the "expected." The "amount of damage" concept is illustrated graphically by the unshaded portions of the columns up to a theoretical "ideal" weight, represented by a horizontal line drawn at the highest average. Certainly, the strongest plants are those with the greatest length of nematode-free portions of roots, and the weakest are those with the least.

Having the complete Maui data available, it has seemed desirable to go still further with their analysis. The following partial correlations were calculated:  $r_{ai,b} = .57$ ;  $r_{ae,b} = .11$ ;  $r_{ie,b} = -.384$

Whence,

$$r_{aib} = \frac{r_{ai,b} - (r_{ae,b} \times r_{ie,b})}{\sqrt{1 - r_{ae,b}^2} \times \sqrt{1 - r_{ie,b}^2}} = .67 \pm .034$$

In other words, the correlation of weight upon length of the portion of roots free from root knot with the very irregularly distributed number of roots and number of galls constant, is considerably higher than the simple correlation,  $r_{ai} = .60$ .

One may go even so far as to calculate the multiple correlation of plant weight on the combined variables, length of healthy root, number of roots, and number of galls.

$$R_{a(ibe)} = \sqrt{1 - (1 - r_{ab}^2)(1 - r_{ae,b}^2)(1 - r_{aib}^2)} = .721$$

The weights that might be predicted by means of the regression equation involving the measurements  $i$ ,  $b$ , and  $e$ , would correlate with the actual weights to this extent.

All of this shows clearly that complex interrelations exist between the different factors, and that all together certainly combine to influence plant weight to an important extent. There is still evident, however, some unmeasured factor that has considerable weight. This may be the deterrent effect of heavy early infection on new root development later in the growth of the plant, mentioned heretofore as a probability. Again, it may be related to the inconsistencies in the gall-count variable in general. It is probably both together with some other unmeasured factor or factors.

Further light may be thrown on the gall-count factor by the calculation of an additional partial correlation.

$$r_{ae,bi} = \frac{r_{ae,b} - (r_{ai,b} \times r_{ie,b})}{\sqrt{1 - r_{ai,b}^2} \times \sqrt{1 - r_{ie,b}^2}} = .434$$

A correlation as high as this between plant weight and gall count, with other factors constant, again appears anomalous, showing as it does a definite

positive influence of some kind between the two variables. Again one must apply (as per Garrett (2)) the principle of common sense to the interpretation. The increased plant growth is not due to increased number of galls, but rather is the increased number of galls due to increased root area subject to attack. In a heavily infested area, by virtue of the potentially rapid rate of multiplication of nematodes (6, p. 425) the "saturation point" may be reached in the root systems of some of the plants at 8 months of growth regardless of the differences in rate of growth of different plants up to that time. That plant that has escaped early heavy infestation has attained greater size, and with it, greater inherent capacity for harboring nematodes than has the less vigorous plant. More large branch roots are pushed out, making opportunity for more galls. The roots have greater power to overcome a terminal infestation, thus making for more of the "non-terminal" galls. In figure 2, in which plant weight and total gall count are compared, 41 of the 140 plants are above average both in number of galls and in weight, and 43 are below the average in both. This accounts for the positive correlation between the two and also explains the unreliability of direct gall count as a measure of the true extent of pathogenic effect involved up to this stage of growth.

The earlier data of the Oahu experiment show higher figures throughout for the really significant "cause and effect" correlations. Unfortunately, the raw data are not available for a similar complete study of correlations. Those derived, however, illustrate the important part played by the length of functioning root on plant weight ( $r, .76$ ) and on yield ( $r, .67$ ). At this earlier reading, the gall count on the percentage basis shows a rather high negative correlation with root length ( $r_{cg} = -.69$ ). The earliest nematode reading of all, the indicator plant reading, correlates to the extent of  $-.852$  with the yield variable on the plot basis. This would seem to indicate that readings for the purpose of measuring the nematode factor must be taken early, before the various vitiating factors have had time to act, in order to have any significant reliability.

Yield is the ultimate criterion as to effect on the plant, inasmuch as it is the resultant of all the other individual effects. In both tests herein reported, the effect of nematode infestation on yield was more striking than any other individual measured effect.

#### GENERAL CONSIDERATIONS

There is much experimental and observational evidence that other species of plants are subject to more extreme injury from root-knot nematode populations of equal magnitude than is the pineapple plant. In other words, the pineapple is definitely rather highly tolerant of nematodes. In the case of the Maui area herein discussed, cowpea plants grown in the soil from the

failing area were either killed or greatly reduced in growth in only 8 weeks' time, as shown by figure 1. The pineapple plants, while greatly damaged prior to 8 months of growth, at least continued to grow, and bore a crop of fruit. In a nematode-control experiment heretofore reported (7) tomato plants showing an average gall count (primary infestation only) of 665, indicating a nematode population in the soil of about 15,000 per cubic foot (3), disclosed the extreme retardation in plant growth to only 1/7 that of the healthy plants in 30 days of growth. This population is about 1/10 that postulated in another paper (6) as necessary seriously to retard the early growth of the pineapple plant. It is about the same as the maximum soil infestation used in a pineapple inoculation experiment also reported in that paper, in which pineapple growth was not visibly retarded after 6 months of growth.

#### SUMMARY

Statistical studies are here reported on pineapple plants growing in two different localities in the Hawaiian Islands, where initial nematode, *Heterodera marioni*, infestations in the soils covered a wide range of intensities.

The studies included measurements of the magnitude of plant infestation on the basis of root-gall counts, and relative thereto counts of numbers of roots, various root and plant-growth measurements, and finally yield of fruits.

In general, while obviously large and important ultimate differences between infested and healthy areas were evident in numbers of roots, lengths of root systems, weights of plants, and yields, the complex interrelations of the various factors and seeming inconsistencies in root-gall counts made the actual correlations between some of the growth measurements and gall counts rather low.

Data taken at 5 months of plant growth showed correlations as follows: Root length with gall count as percentage of total roots showing terminal galls,  $-.69$ ; plant weight with root length,  $.76$ ; length of roots with number of roots,  $.61$ . The correlation of fruit yield with root length at 5 months was  $.67$ ; yield with original nematode population as determined by indicator plant readings,  $-.85$ .

In data taken at 8 months of growth in another location, the corresponding correlations were lower throughout.

Direct gall count in the later reading was completely unreliable as a measure of nematode injury to the plants. In fact there was a positive correlation of  $.26$  between gall count and plant weight. The increased vigor of the heavier plants, derived from early escape from heavy infestation, is indicated as being responsible for the higher gall count later, due to increased

root surface exposed to infestation in the virtually "nematode-saturated" soil.

The same identical population of plants showed a positive correlation of .57 between length of roots and plant weight. The root system that develops in spite of nematodes, therefore, is more closely correlated with plant growth in a positive way than is the gall count by any of the criteria tested in a negative way.

The multiple correlation of plant weight on the combined factors of root number, root length, and number of galls was .72.

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## AN EXPERIMENTAL STUDY OF SOME FUNGI INJURIOUS TO SEEDLING FLAX

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The importance of *Fusarium lini* Bolley in decreasing the stand of flax when successive crops are grown on the same soil has been long known, and its importance in the United States as the principal agent of flax degeneration has led to the comparative neglect of the possibility of other soil organisms as factors in the development of a "flax sick" soil. That fungi other than *F. lini* can attack flax has been demonstrated by Bolley and Manns (4), Brentzel (6), and Boyle (5) in the United States, and by many workers in other regions, especially Europe.

Attempts to control flax wilt by selection of varieties resistant to *Fusarium lini* have been successful, but it is known that some wilt-resistant varieties, after a few years of cultivation, did not live up to their initial promise. That the change in behavior is not due, as was at one time thought by Bolley (3), to a real loss of resistance was demonstrated by Barker (2). The work of Broadfoot (7), indicating the presence of physiologic races, furnished at least a possible partial explanation for the wilting of supposedly resistant varieties.

Many other fungi, including *Colletotrichum lini* (Westerdijk) Tochinai, *Thielavia basicola* Zopf, *Asterocystis radialis* de Wildemann, *Pythium megalacanthum* de Bary and other *Pythium* spp., have been recorded in several countries as causal agents of root injury in flax plants (1, 8, 9, 12, 16, 17, 20, 21, 22, 23, 28). The possibility that some of these fungi play a part in the degeneration of reputedly resistant flax varieties should not be overlooked, and it was, therefore, the purpose of this investigation to ascertain to what degree flax in Minnesota is susceptible to fungi other than *Fusarium lini*.

No attempt will be made to discuss the literature on flax wilt caused by *Fusarium lini*, since that has been reviewed adequately in a number of papers (2, 11, 28). A general review of flax diseases is given by Schilling, in Tobler's "Der Flachs als Faser- und Ölpflanze" (28).

<sup>1</sup> The investigation was carried out in the Department of Plant Pathology, University of Minnesota, during my tenure of a Commonwealth Fund Fellowship. It gives me great pleasure to acknowledge my indebtedness to Dr. E. C. Stakman and members of the Department of Plant Pathology, University of Minnesota, for their interest and assistance during the course of the experimental work.

## MATERIAL AND METHODS

The purpose of this investigation being an attempt to find what fungi other than *Fusarium lini* are capable of causing injury to flax roots, cultures were obtained from as many sources as possible. Isolations were made from samples of seed collected throughout Minnesota, the majority of the cultures obtained being *Alternaria*; *F. lini* and several nonpathogenic *Fusaria* were next most frequent. Several nonpathogenic fungi were obtained, some of which were identified.

Fungi attacking the roots or associated with them were obtained from material growing in the field and also from seedlings grown in the greenhouse in soil collected from different localities in Minnesota. Most commonly found in the roots was *Fusarium lini*; two isolations of *Helminthosporium* were obtained, one—a *Brachysporium* type, the other, *H. sativum*. *Rhizoctonia solani* was isolated twice and *Thielavia basicola* once. Numerous nonpathogenic forms, frequently isolated with *F. lini*, were obtained. Of cultures obtained by direct isolation from the soil, only *F. lini* and a species of *Pythium* were strongly pathogenic. An isolate of *Trichoderma lignorum* (Tode) Harz. caused slight injury, but no others were pathogenic. Several fungi from other hosts were obtained through the courtesy of workers at the Minnesota Experiment Station.

Preliminary pathogenicity tests made possible the elimination of many of the cultures. Only the results obtained with cultures, pathogenic in the preliminary tests or related to forms noted in the literature as pathogenic, will be reported in this paper.

One variety of flax, Winona, was used in the tests. The plants were grown on steamed soil plus inoculum, the fungus being grown in Erlenmeyer flasks containing a medium consisting of a mixture of 50 g. five per cent corn meal in sterile soil and 20 cc. of water per flask. To the control pots were added equivalent amounts of this medium. Twenty-five seeds were sown in each pot, there being 4 replications in each experiment.

## PATHOGENICITY OF CERTAIN FUNGI ON FLAX

*Helminthosporium* spp.

Two species of *Helminthosporium* associated with flax have been recorded. Gentner (10), in Bavaria, found on a few samples of flax seed a species that he considered new. He named it *Helminthosporium lini*. In 1929 Kletschetoff (18) described another species, *H. linicoli*, which he found occurring on flax roots and capable of infecting young flax seedlings.

Two isolates of *Helminthosporium* ( $H_6$  and  $H_7$ ) were obtained from diseased roots of Winona flax grown in the greenhouse on flax-sick soil obtained



from the University Farm, St. Paul. Both of these isolates were capable of causing extensive injury (Table 1):  $H_7$  apparently caused more preemergence injury than  $H_6$ , and, in all, was capable of causing more injury to the plants.  $H_7$  resembled the general type of *H. sativum* P. K. and B., while  $H_6$  fell into the *Brachysporium* group.

TABLE 1.—*The pathogenicity of Helminthosporium isolates for flax seedlings*

Culture number <sup>a</sup>	Isolated from	Classification	Number of plants emerged 6 days after planting	Number of survivors 22 days after planting	Average height (cms.) of plants after 22 days	Percentage diseased plants after 22 days
$H_1$	Barley	Resemble <i>H. tetramera</i> McKinney	78	92	13.5	0
$H_{11}$	Barley		66	72	14.0	5.5
$H_2$	Wheat		20	48	11.0	100
$H_{15}$	Poa	Resemble Helminthosporium N group.	68	84	13.0	4.0
$H_{16}$	Barley	(See Henry (12).)	64	70	13.0	17.1
$H_{18}$	Wheat		76	86	14.0	0
$H_{19}$	Poa		82	88	13.5	0
$H_3$	Barley		76	84	14.0	0
$H_6$	Flax roots	<i>Brachysporium</i> type	80	84	13.5	73.8
$H_8$	Oats		70	82	14.0	0
$H_9$	Barley		78	80	14.0	5.0
$H_4$	Rye		72	74	12.0	62.2
$H_7$	Flax roots	<i>H. sativum</i> P. K.	70	64	9.5	78.1
$H_{10}$	Barley	and B	80	76	14.0	5.0
$H_{17}$	Barley		50	62	9.0	77.4
$H_5$	Barley	Intermediate between <i>H. sativum</i> and <i>H. tetramera</i>	70	72	13	0
$H_{14}$	Barley		76	84	14.0	0
$H_{20}$	Poa		68	70	15.0	0
$H_{21}$	.....	<i>H. monoceras</i> Drechsler	88	88	13.0	0
$H_{13}$	Barley	Apparently undescribed species	48	72	11	66.6
Check			80	82	13.5	0.0

<sup>a</sup> All of the isolates described above, with the exception of  $H_6$  and  $H_7$ , were obtained from J. J. Christensen, who also kindly supplied the information in regard to their origin and classification.

To determine if isolates of *Helminthosporium* from cereals and grasses might be pathogenic, 19 strains falling into 7 distinct species were obtained through the courtesy of J. J. Christensen. From table 1 it can be seen that 4 of them,  $H_2$ , isolated from wheat and belonging to the *Helminthosporium* N type described by Henry,  $H_4$  from rye, and  $H_{17}$  from barley, both belonging to *H. sativum*, and  $H_{13}$  from barley, an apparently undescribed species,

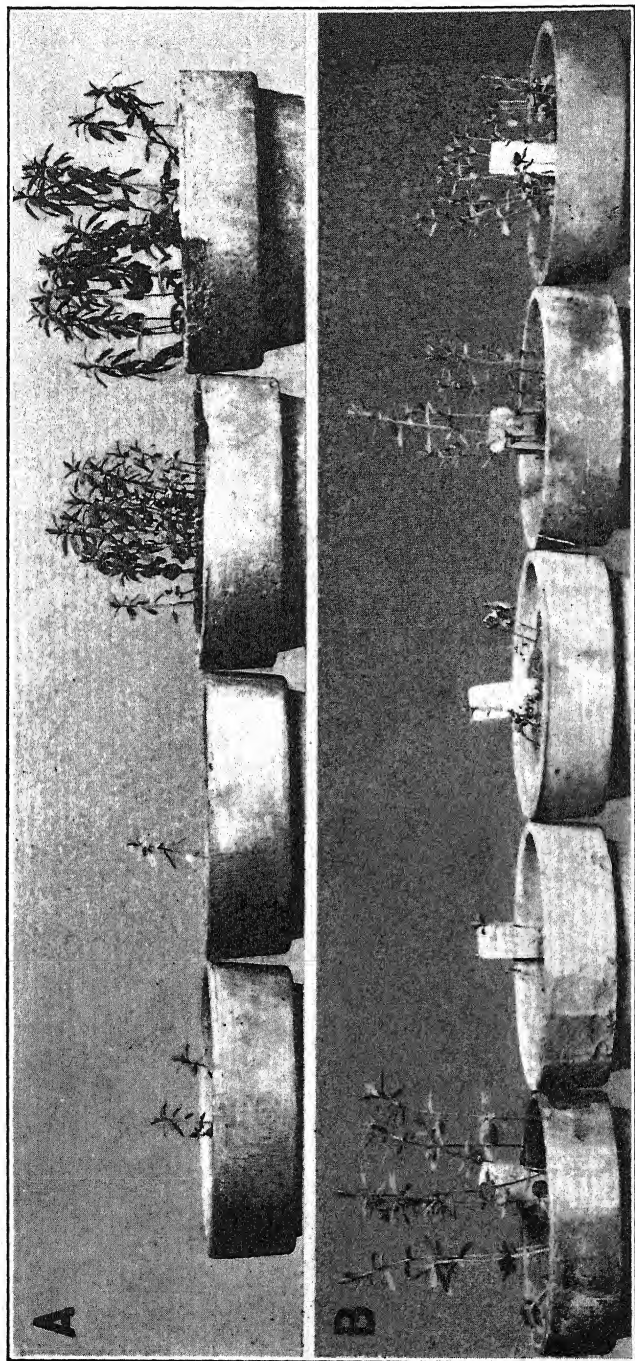


FIG. 1. A. Relative pathogenicity of *Helminthosporium* isolates. Left to right:  $H_2$ ,  $H_4$ ,  $H_5$ , check. B. Relative virulence of *Fusarium lini* and *Helminthosporium sativum* in peat and in steamed soil. Left to right: Check in peat; *F. lini* in peat; *F. lini* in steamed soil; *H. sativum* in peat; *H. sativum* in steamed soil.

were very pathogenic, causing infection ranging from 100 per cent in the case of  $H_2$  to 62 per cent in the case of  $H_4$ . These strains were as virulent as  $H_6$  and  $H_7$ , the two isolated from flax. (Figs. 1, A and 2.)

The symptoms produced by these fungi differed somewhat. Strains  $H_2$ ,  $H_7$ ,  $H_{17}$ , and, under certain conditions, strain  $H_4$  (Table 2), did considerable

TABLE 2.—*Effect of temperature on pathogenicity of Helminthosporium isolates to flax seedlings*

Culture number	Time of experiment	Approximate temperature of greenhouse	Percentage seedlings killed
$H_2$	Oct. 29 to Nov. 19	70° F	45.3
$H_4$	do	do	0
$H_6$	do	do	0
$H_7$	Nov. 17 to Nov. 30	do	45.5
$H_2$	Feb. 4 to Feb. 28	80° F.	60.2
$H_4$	do	do	85.9
$H_6$	do	do	33.3
$H_7$	do	do	50.0
$H_2$	Apr. 19 to May 11	50 to 80° F.	41.4
$H_4$	do	do	9.7
$H_6$	do	do	0
$H_7$	do	do	21.9

preemergence damage,  $H_2$  and  $H_{13}$  delaying germination somewhat more than the others, although in all cases the plants were stunted to some extent. The root system was always severely attacked, in most cases with complete destruction of the tap root, the absorptive system being restricted to secondary roots that had developed just below the ground level. The plants were all chlorotic to a greater or less degree, the lower leaves rapidly turning yellow and dying prematurely. This in particular was more typical of the attack of  $H_4$  than of the others; under the conditions during which this experiment was run, the plants attacked by  $H_4$  were quite tall, averaging 12 cm. as compared with 13.5 cm. for the controls, but the plants were very spindly and chlorotic.

During the course of a year 4 of the strains,  $H_2$ ,  $H_4$ ,  $H_6$ , and  $H_7$ , were tested at 3 different times. Table 2 gives a summary of the results obtained and shows the variability in the effect of these cultures caused by environmental conditions. In the fall the greenhouse was kept at about 70° F., and there seedlings were not killed by  $H_4$  and  $H_6$ , but 45 per cent were killed by  $H_2$  and by  $H_7$ . During the winter this series of cultures was tested in a greenhouse kept at about 80° F., and at that time  $H_4$  not only killed more seedlings than the others but also caused much more stunting than  $H_2$ . The

experiment was repeated in April, when the temperature in the greenhouse was quite variable owing to fluctuating external conditions. In general, the temperature was below 80° F., ranging from about 50° F. to 80° F., and, on occasion, higher. In this experiment the percentages of seedling killing fairly closely corresponded to those in the fall.

*Rhizoctonia* spp.

Brentzel (6), in 1923, made note of the occurrence of *Rhizoctonia* on flax in eastern North Dakota, where it caused considerable damage, the plants taking on the general appearance of wilt. Thomas (27) found that 2 species of *Rhizoctonia*, *R. psychodis* and *R. suavis*, both orchid endophytes, attacked flax.

In the experiments to be described (Table 3), 16 isolates of *Rhizoctonia*, 12 of *R. solani*, one of *R. baticola*, the other 3 from flax, not definitely identified, but approximating the general *R. solani* type, were tested on flax. Of these isolates, 10 caused extensive seedling killing, 50 to 100 per cent, the other 6 causing less than 30 per cent injury. The most virulent cultures were isolated from eggplant, tomato, flax, barley seed, pea, bean, and sugar beet. Most injury occurred in the preemergence stage, frequently only a very few plants emerging at all. In cases where the attack was not sufficiently severe to prevent emergence, the plants were generally dwarfed, and there was a high degree of root injury, the tap root being most frequently attacked and usually rotted through a short distance below ground level. Depending on the virulence of the strain, secondary roots sometimes developed, the plants sometimes remained healthy in appearance and recovered from the attack, although they generally were stunted to some degree.

LeClerc (19) found that the *Rhizoctonia* root rot of sugar beets is caused by strains of *R. solani* distinct from those obtained from potato. That flax might be susceptible to only one group of these isolates was thought possible; therefore, 10 cultures of *R. solani* from sugar beet and 10 from potato, which had been used by LeClerc in his experiments, and were furnished by him, were tested against flax. The results (Table 4) confirm the observations of LeClerc as to the existence of 2 groups, separable on the basis of their pathogenicity. Six of the sugar-beet isolates caused 60 per cent or more damping off of Winona flax plants, while none of the potato isolates caused any. Only one potato strain was capable of doing much damage to the roots; but, even in this case, the plants germinated well and were only slightly stunted; 5 of the potato strains damaged the roots, from 10 to 65 per cent of the plants showing signs of injury.

*Thielavia basicola* Zopf

*Thielavia basicola* has been recorded on flax from the United States (3), Ireland (23), Germany (25), Russia (17), and Holland (9); but, in general,

TABLE 3.—Results of damping-off tests with flax caused by isolates of *Rhizoctonia*

Culture number <sup>a</sup>	Host	Location	Isolator	Duration of experiment	Number survivors	Percentage damping off <sup>b</sup>
					Number controls	
MR <sub>1</sub>	Tomato	Baton Rouge, La.	L. H. Person	May 1 to 14	0 81	100
MR <sub>12</sub>	Egg-plant	do	do	do	0 81	100
MR <sub>25</sub>	Flax	Fargo, N. D.	H. H. Flor	Apr. 16 to 26	3 79	96.2
MR <sub>7</sub>	Pea	Moscow, Idaho	L. H. Person	May 1 to 14	3 81	96.3
MR <sub>75</sub>	Flax	St. Paul, Minn.	I. W. Tervet	Apr. 12 to 19	5 92	95.6
MR <sub>18</sub>	Barley seed	do	J. J. Christensen	May 1 to 14	7 81	91.4
MR <sub>16</sub>	Bean	Houma, La.	L. H. Person	do	8 81	90.1
SB <sub>12</sub>	Sugar beet	Michigan	E. L. LeClerc	Apr. 17 to 26	21 79	73.4
SB <sub>13</sub>	Sugar beet	Chaska, Minn.	do	May 1 to 14	35 81	56.8
MR <sub>11</sub>	Rice	Welch, La.	L. H. Person	do	39 81	51.8
MR <sub>74</sub>	Flax	St. Paul, Minn.	I. W. Tervet	Apr. 6 to 25	54 75	28.0
MR <sub>5</sub> <sup>c</sup>	Sugar cane	Baton Rouge, La.	L. H. Person	May 1 to 14	60 81	25.9
P <sub>38</sub>	Potato	Dove Creek, Colo.	E. L. LeClerc	Apr. 16 to 26	62 79	21.5
MR <sub>4</sub>	Sugar cane	Baton Rouge, La.	L. H. Person	May 1 to 14	69 81	14.8
P <sub>48</sub>	Potato	Scott's Bluff, Nebr.	E. L. LeClerc	Apr. 16 to 26	73 79	7.6
P <sub>11</sub>	Potato	Presque Isle, Maine	do	Nov. 6 to 19	77 68	0

<sup>a</sup> The culture numbers given above agree with the classification used by E. L. LeClerc for his stock cultures of *Rhizoctonia*.

<sup>b</sup> The percentage damping off is estimated by assuming the check pots to be 0 per cent damping off.

<sup>c</sup> This culture is an isolate of *R. baticola*; all others are *R. solani*.

TABLE 4.—Results of damping-off tests with flax to compare the relative pathogenicity of isolates from sugar beet and potato

Sugar-beet isolates				Potato isolates			
Culture number	Location	Percentage plants		Culture number	Location	Percentage plants	
		Damped off <sup>a</sup>	Diseased <sup>b</sup>			Damped off <sup>a</sup>	Diseased <sup>b</sup>
SB <sub>37</sub>	Michigan	100	100	P <sub>14</sub>	Dilworth, Minn.	0.0	63.5
45	California	100	100	22	do	0.0	33.8
39	Michigan	94.5	98.6	7	Grand Forks, N. D.	0.0	27.0
49	do	91.9	98.6	11	Glyndon, Minn.	0.0	10.8
42	do	81.1	94.5	13	St. Agatha, Manitoba	0.0	10.8
13	Chaska, Minn.	60.8	64.9	12	do	0.0	0.0
43	Ohio	29.7	41.9	23	Dilworth, Minn.	0.0	0.0
18	Mankato, Minn.	24.3	27.0	3	Guthrie, Minn.	0.0	0.0
23	do	22.9	22.9	18	Moorhead, Minn.	0.0	0.0
28	do	0.0	0.0	4	Fosston, Minn.	0.0	0.0

<sup>a</sup> In estimating the percentage damping off, the survival rate in the check pots is taken as 100 per cent.

<sup>b</sup> In estimating the percentage of diseased plants, at the end of the experiment all plants that had diseased roots were included along with those plants killed prior to the conclusion of the test. Again, the check pots were assumed to have a survival rate of 100 per cent, and no diseased roots were observed in any of the controls.

it is not recognized as a very virulent pathogen of flax. Johnson (14) was unable to cause infection with it, although a number of workers (13, 17, 23, 25) have demonstrated that it is capable of causing injury to the roots, and Boyle (5) was able to demonstrate its presence in roots of flax. In Holland it is mainly considered to be of secondary importance to *Pythium megalarcanthum*. The writer isolated *T. basicola* from roots of Winona flax on one occasion only and under favorable conditions found it capable of causing marked stunting, with frequently a severe rot of the tap root and the production of extensive red and black lesions on the roots.

*Ophiobolus cariceti* (Berk. and Broome) Sacc.

Although *Ophiobolus cariceti* is considered to be restricted to members of the Gramineae (15), in experiments here it has attacked flax. One cul-

ture from wheat, furnished by F. R. Davies, was found capable of producing red lesions, frequently very elongated; the tap root was occasionally completely rotted and the lateral roots often severely attacked, especially close to the growing point.

*Alternaria* sp.

Ninety-two isolates of *Alternaria* were obtained, 84 from seeds and 8 from roots of flax. None of the root isolates caused injury, and they probably were secondary fungi associated with *Fusarium lini*, since, in many isolations from the roots of wilted plants, both *F. lini* and *Alternaria* sp. were obtained. Twenty-one isolates from seeds were tested, but only 5 were able to infect the roots, and only 2 caused appreciable damage. Here the infection was evident by a stunting of the plants and damage to the root system, either by an actual limitation of development or by destruction of the tap root. Repetition of the experiments with one of the parasitic isolates did not always yield the same result, due probably to different environmental conditions (Table 5). The attack under the most favorable conditions for infection resulted in a very marked stunting of the root system, usually with destruction of the tap root; with conditions less favorable for the production of disease, the injury to the roots was confined to the formation of reddish lesions, mainly on the tap root.

Bolley and Manns (4) found an *Alternaria* killing flax, and Gentner (10) records one, isolated from seed, that reduced germination. Several of the isolates obtained from seed by the writer caused some reduction in germination as compared with the controls.

*Pythium* spp.

Of species of *Pythium* recorded in the literature as attacking flax, *P. megalacanthum* deBary is considered the most destructive, being reported as the sole cause of "Vlasbrand" or scorch in Holland (9). *P. deBaryanum* Hesse and *P. irregulare* Buisman also caused severe infection, but the symptoms resembled damping off rather than scorch.

One culture isolated by the writer from wilt-sick soil caused severe damping off, only 4 plants out of 100 surviving 13 days after planting. One isolate from bean was equally pathogenic, but 2 others, 1 from sugar cane and the other from potato, were not pathogenic.

*Phytophthora* spp.

Stevens and Plunket (26), in 1925, found that strains of *P. cactorum* from tulips caused a damping off of inoculated seedlings of flax. Five isolations of *Phytophthora*, *P. parasitica* Dast., *P. cryptogea* Pethybr., *P. paeoniae* Cooper and Porter, *P. cactorum* (L. and C.) Schroet., *P. fagi* (Hart), were tried by the writer, but none caused any injury to flax.

TABLE 5.—Effect of different soils on the pathogenicity of six fungi for flax seedlings

Organism and culture number	Number of plants emerged after 7 days				Height of plants (in centimeters) after 32 days					Percentage of diseased plants <sup>a</sup>			
	Number of plants surviving after 32 days				Steamed soil	Corn soil	Prairie soil	Peat soil		Steamed soil	Corn soil	Prairie soil	Peat soil
	Steamed soil	Corn soil	Prairie soil	Peat soil									
<i>Aminthosporium sativum</i> , H <sub>4</sub>	81 80	72 63	74 72	35 32	14	19 (10 plants) 10 (53 do )	19	9		98.8	73.6	97.3	82.9
<i>Helveta basicola</i> .....	81 57	46 45	76 77	42 40	20	17	20	20 (36 plants) 9 ( 4 do )		67.2	35.1	41.0	21.1
<i>Hibobolus variceti</i> .....	81 57	62 62	78 75	49 48	20	21	19	18 (42 plants) 13 ( 6 do )		73.8	48.0	58.2	57.4
<i>Ernaria</i> sp.	77 53	79 75	69 66	42 43	20	20	21	21 (39 plants) 9 ( 4 do )		37.9	27.3	18.6	9.5
<i>Isotonia olani</i> , MR <sub>4</sub>	68 52	60 54	70 68	46 45	18	20 (46 plants) 10 ( 8 do )	17	19		10.9	45.3	11.5	17.2
<i>Varium ni</i> , W <sub>4</sub> .....	63 1	57 0	66 1	34 6	.....	.....	.....	.....		100.0	100.0	100.0	95.3
<i>Urol</i> .....	75 67	25 23	68 68	42 41	22	22	21	21		16.1	12.0	0.0	25.0

<sup>a</sup> The percentage of diseased plants includes all that died before the conclusion of the experiment plus plants that remained alive but showed definite signs of infection on the root system.



EFFECT OF DIFFERENT SOILS ON THE PATHOGENICITY OF SIX FUNGI PARASITIC  
ON FLAX

As a result of the foregoing studies, 6 cultures were selected for test on 3 different soil types. The cultures were *Helminthosporium sativum* ( $H_4$ ) from rye, *Thielavia basicola* isolated from flax roots, *Ophiobolus cariceti* from wheat, *Alternaria* sp., isolated from flax seed, *Rhizoctonia solani* ( $MR_{74}$ ) from flax, and a very virulent strain of *Fusarium lini* ( $W_{41}$ ).

The fungi were grown on the standard corn-soil medium and the different soils infested with the inoculum. The experiment was replicated 4 times, 25 seeds of Winona being planted in each pot. The 4 soils used were: (1) a type designated as "corn soil," a sandy loam, which was obtained from the University Farm, St. Paul, and which, in 1934, had been under cultivation to corn; (2) a prairie soil obtained from the vicinity of Fort Snelling, Minnesota, and, so far as is known, never cultivated; (3) a peat soil from the University Experimental Farm at Anoka, Minnesota, on which flax had been grown the previous 2 years; (4) steamed soil, which was the normal potting soil for the greenhouse and consisted of equal parts of loam and sand.

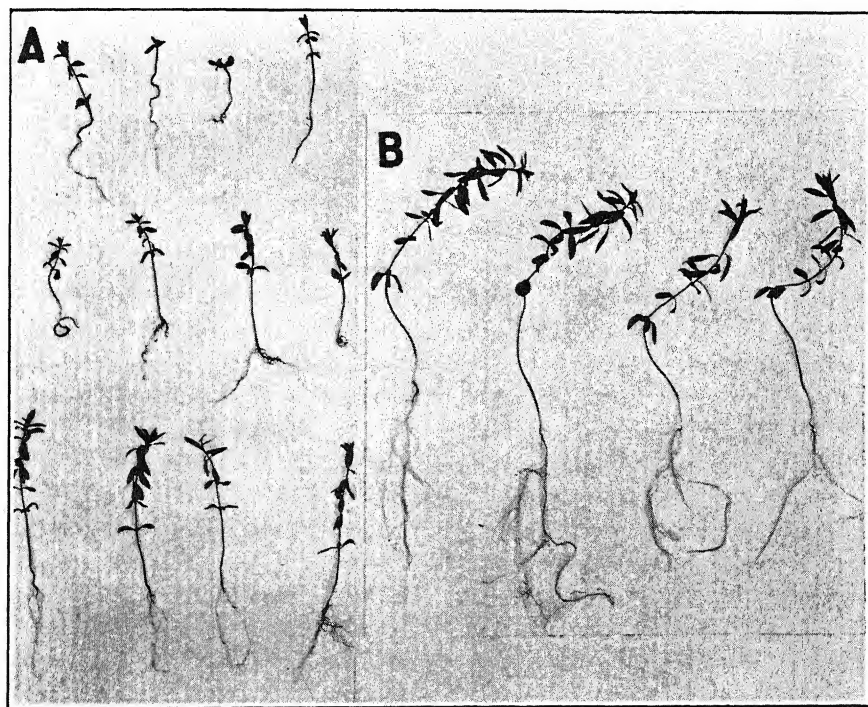


FIG. 2. Root development influenced by activity of *Helminthosporium* isolates. A. Top to bottom:  $H_2$ ,  $H_4$ ,  $H_7$ . B. Check.

From table 5 and figures 1, B, 3, A, and 3, B, it can be seen that *Fusarium lini* is very virulent throughout, with *Helminthosporium sativum* almost as much so. There was considerable variation in the appearance of the plants attacked by *H. sativum*. In steamed soil the plants were stunted, with the lower leaves quickly dying. In the corn soil and the peat soil the stunting was much more marked, the plants in the peat being less than half the height of the controls; and in the corn soil the majority were almost as small, with about 20 per cent of the plants slightly attacked and correspondingly more vigorous. In the prairie soil the plants were very tall and spindly, presenting quite a different appearance from the rest of the plants infected with *H. sativum*. They were undoubtedly heavily infected, the root systems of 97 per cent of the plants being attacked.

*Ophiobolus cariceti* also caused considerable injury, although only in steamed soil was there much seedling killing. In the remainder, although up to 58 per cent of the roots were infected, little actual killing had occurred at the end of 32 days. The plants were not stunted or but slightly so. With *Thielavia basicola* again the greatest injury occurred in the steamed soil, while with *Alternaria* and *Rhizoctonia solani* the results were rather inconsistent.

#### EFFECT OF FUNGI ON FLAX IN THE FIELD

Since the greenhouse studies indicated that certain fungi were pathogenic to flax seedlings and other fungi appeared to exert an antibiotic effect on the pathogenic fungi, field experiments were made in the spring of 1935. Plots 6 x 5 feet, and 2 feet apart, were artificially inoculated with various fungi, alone or in combination. The inoculum used was prepared by growing the fungi on sterilized oat hulls in flasks and jars. The fungi used in the field experiments were *Rhizoctonia* spp., *Fusarium lini*, *Helminthosporium sativum*, *Thielavia basicola*, *Chaetomium* spp., and *Trichoderma lignorum*. Seed of Winona flax was sown in 6 5-foot rows in each plot soon after the soil was artificially inoculated. In none of the plots was there either seedling or root injury at any stage of their development, although occasional mature plants throughout the plots wilted. Since the plants in plots infested with a virulent strain of *Fusarium lini* were not injured more than those in the check plots, it was undoubtedly true that insufficient inoculum was applied or insufficient time was allowed for the pathogen to establish itself in the soil. Other factors, such as temperature and condition of the soil, also might have prevented seedling and root injury.

#### DISCUSSION AND CONCLUSIONS

The concept that a "sick soil" is the result of the cumulative effects of certain soil pathogens generally is accepted at the present day, the former

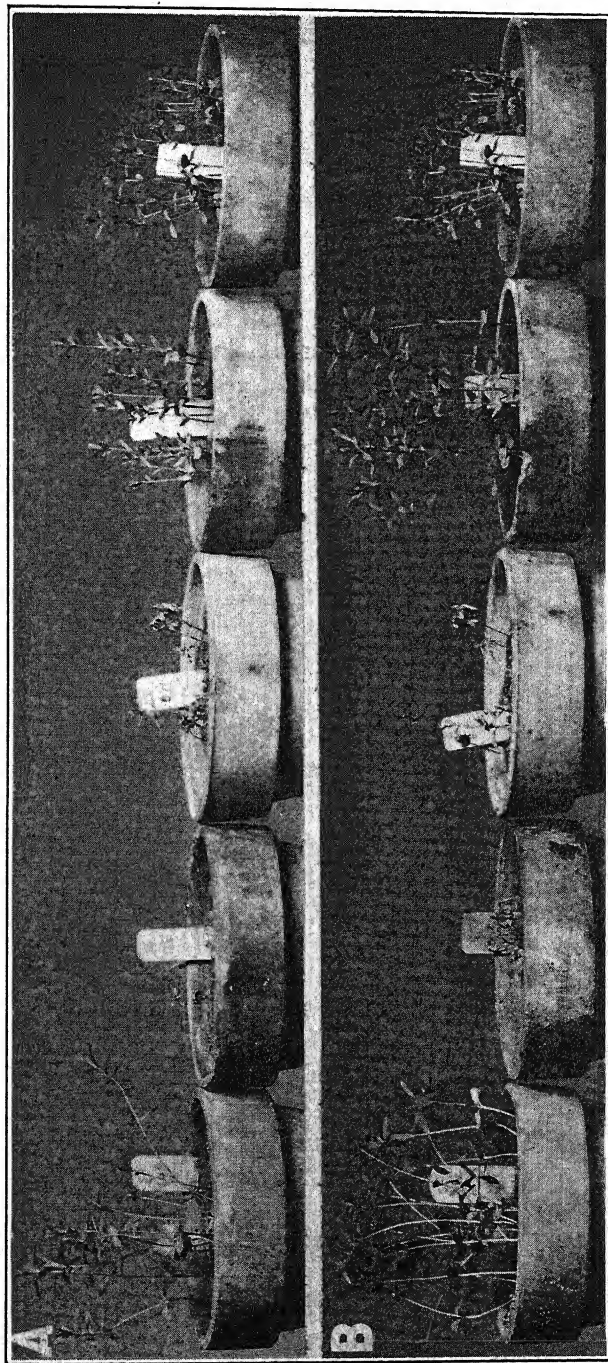


FIG. 3. A. Relative virulence of *Fusarium lini* and *Helminthosporium sativum* in sandy loam and prairie soil. Left to right: Check in sandy loam; *F. lini* in sandy loam; *F. lini* in prairie soil; *H. sativum* in sandy loam; *H. sativum* in steamed soil. B. Relative virulence of *F. lini* W<sub>4</sub> and *H. sativum* H<sub>4</sub> in prairie and in steamed soil. Left to right: Check in prairie soil; *F. lini* in prairie soil; *F. lini* in steamed soil; *H. sativum* in prairie soil; *H. sativum* in steamed soil.

ideas of soil depletion and the production of autotoxins by the plants being practically discarded. That fungi other than *Fusarium lini* are capable of injuring flax is, of course, not a new idea. As long ago as 1901 Bolley and Manns (4), in North Dakota, obtained evidence of the pathogenic action of certain fungi local to that region as well as others introduced on imported seed. *Colletotrichum lini*, although noted by Bolley and Manns, has not been observed recently and is virtually unrecognized in this region.

The observation by Brentzel (6) of the attack upon flax in the Midwest by *Rhizoctonia* has been amply confirmed in the present investigation. Not only is flax susceptible to strains isolated from that host, but it also was found attacked by a number of forms from several other host plants. Of possibly greatest interest is the observation of the virulence of certain sugar-beet isolates and the much greater resistance shown to the potato strains.

The record, so far as the author is aware, of only 2 isolations of *Helminthosporium* from flax (10, 18), one from seed, the other from the roots, is rather surprising when one considers the virulence shown by some of the strains of *Helminthosporium* tested, not only in steamed soil, but in soils with a presumably active flora. The reisolation of the fungus occasions no difficulty, and, considering the widespread occurrence of the *Helminthosporium* species in soils and on weeds, one is puzzled as to the nonobservance of this fungus in the past. It is difficult to understand why this fungus, little or possibly no less pathogenic than *Fusarium lini*, has not been more frequently observed on flax.

One phase of the disease problem, important because of the possibility of its control by seed treatment, is the preemergence injury that is very evident under certain conditions. Strains of *Helminthosporium*, *Rhizoctonia*, and *Pythium* can cause great damage to the stand, and, as has been shown, may result in a complete killing of the seeds during or immediately following germination. Ruschmann (24) considers that this form of soil sickness, that is, injury at germination, is due to bacterial development on the mucous coating of the seeds, leading to the seeds being less able to absorb water and thereby rendering them more liable to injury. Of necessity this is not always so, as the action of the *Helminthosporium* and *Rhizoctonia* isolates, and to a less degree other fungi, shows that fungi are capable of causing severe injury to the germinating seeds. A contrary view as to the function and value of the mucous coating is expressed by Schilling (25), who considers that the removal of the mucous membrane plays an important part in the elimination of microorganisms.

From the results noted above, which show that fungi belonging to 7 genera are capable of causing varying degrees of injury to seedling flax, the existence of a "soil sickness" complex can be taken as an established fact. These fungi, belonging to widely divergent groups, presumably respond in

their attacks to different environmental conditions and thereby complicate any one means of control. The presence of other hosts in the case of the most virulent parasites, *Helminthosporium* spp. and *Rhizoctonia* spp., suggests that crop rotation may be of limited value. It probably would be difficult to obtain varieties of flax resistant to all of these organisms and it is probable that the lack of resistance shown by certain varieties to wilt (*Fusarium lini*) may be due in part to attack by other fungi, such as certain species of *Helminthosporium*.

#### SUMMARY

Fungi isolated from flax seeds and roots and fungi from other host plants have been tested for pathogenicity on Winona flax.

Severe seedling blight in steamed soil has been caused under greenhouse conditions by 6 strains of *Helminthosporium* belonging to 4 species, 2 of which were isolated from wheat, rye, and barley, and 2 from flax. Some isolates of *Rhizoctonia solani* from sugar beet, flax, and other crops were pathogenic, while isolates from the potato did little damage.

*Thielavia basicola*, *Ophiobolus cariceti*, *Pythium* spp. and *Alternaria* sp. from flax and other host plants were in some cases responsible for seedling blight of flax.

Species of fungi, pathogenic to flax, were introduced into 4 soils of different origin. Observations on the modification of seedling blight of flax in such infected soils are given.

It is suggested that in some manner the loss of resistance to wilt of flax varieties may be due to the attack of fungi other than *Fusarium lini*.

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## SEXUAL FUSION IN USTILAGO AVENAE UNDER NATURAL CONDITIONS

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*Ustilago avenae* (Pers.) Jens. produces sporidia abundantly in culture at temperatures ranging from 5° to nearly 30° C. According to Bartholomew and Jones (2) and Jones (9), they usually do not develop at temperatures higher than 29–30° C., and Hüttig's (8) results in Germany confirm this view. Other environmental factors, such as moisture and concentration of oxygen, may modify the process and Stakman (13) has shown that the amount of available nutrients also influences both the extent and the rate of sporidial production. It is known that the sporidia produced by smut fungi usually are haploid and may be split up into "sexual" or "fusion" groups. Up to the present only 2 such groups have been found in *U. avenae*. Fusions take place between sporidia of opposite sex and these give rise to the dikaryotic infection hyphae, which alone are capable of penetrating and infecting the host plant. The fact that infection could be brought about by inoculation with sporidia from culture was demonstrated by Brefeld (4) and Lutman (11), both of whom described the penetration of the host epidermis by germ tubes arising from sporidia lodged on the surface. This has been confirmed many times, but it is now known that infection of the host plant results only if the inoculum consists of a mixture of sporidial lines of opposite sex. Monosporidial lines alone, as far as the oat smuts are concerned, cannot cause infection.

The fact that sporidia are produced so readily in culture possibly has led to an overestimation of their importance under natural conditions. In nature, initial infection of the host by *Ustilago avenae* is brought about at flowering time by means of chlamydospores that are blown into the open florets and there lodge upon the stigmas or between the glumes. The spores germinate upon the stigmas and upon the inner surface of the glumes and give rise to mycelia or germ tubes that enter the superficial tissues and there become more or less dormant until the young oat embryo commences to grow, when penetration of the coleoptile and mesocotyl takes place. There has been a difference of opinion as to which source of inoculum is most effective in causing infection. Zade (15, 16) and his coworkers (Arland (1), Diehl (5), and Roesch (12)) hold the view that the resting mycelium within the glumes is chiefly responsible for infection. Gage (6), on the other hand, believes the mycelium that penetrates the style of the flower and hibernates in the pericarp of the seed, and occasionally in the young embryo itself, more often initiates the attack. Diehl (5) also reported the presence of the



fungus in the epidermis of the ovary. For the present purpose the actual location of the inoculum is not so important as its type and origin, *i.e.*, whether the germinating spores produce sporidia or merely germ tubes and where the sexual fusions occur.

It seems to have been the experience of most workers that sporidia are produced but sparingly under natural conditions. Arland (1) found very few sporidia to be produced by spores germinating on the stigmas, but this may possibly have been due to the effect of a rather high temperature. Gage (6) suggests a similar reason for the scarcity of sporidia in his experiments. Diehl (5) obtained sporidia in greater numbers under cool moist conditions than when the weather was dry and warm. Under the latter conditions the spores germinated directly into germ tubes.

In the course of studies upon the initial entrance of smut fungi into oat plants, the present writer observed numerous penetration tubes that could be traced back directly to the chlamydospores, but in no case were such infection hyphae observed arising from sporidia. In view of the importance of the origin of the cells that undergo fusion before the host is invaded, and its effect upon the maintenance of the purity of parasitic races of the pathogen, further information was sought as to the prevalence of sporidial production under natural conditions. The results of these investigations are recorded below.

#### MATERIAL AND METHODS

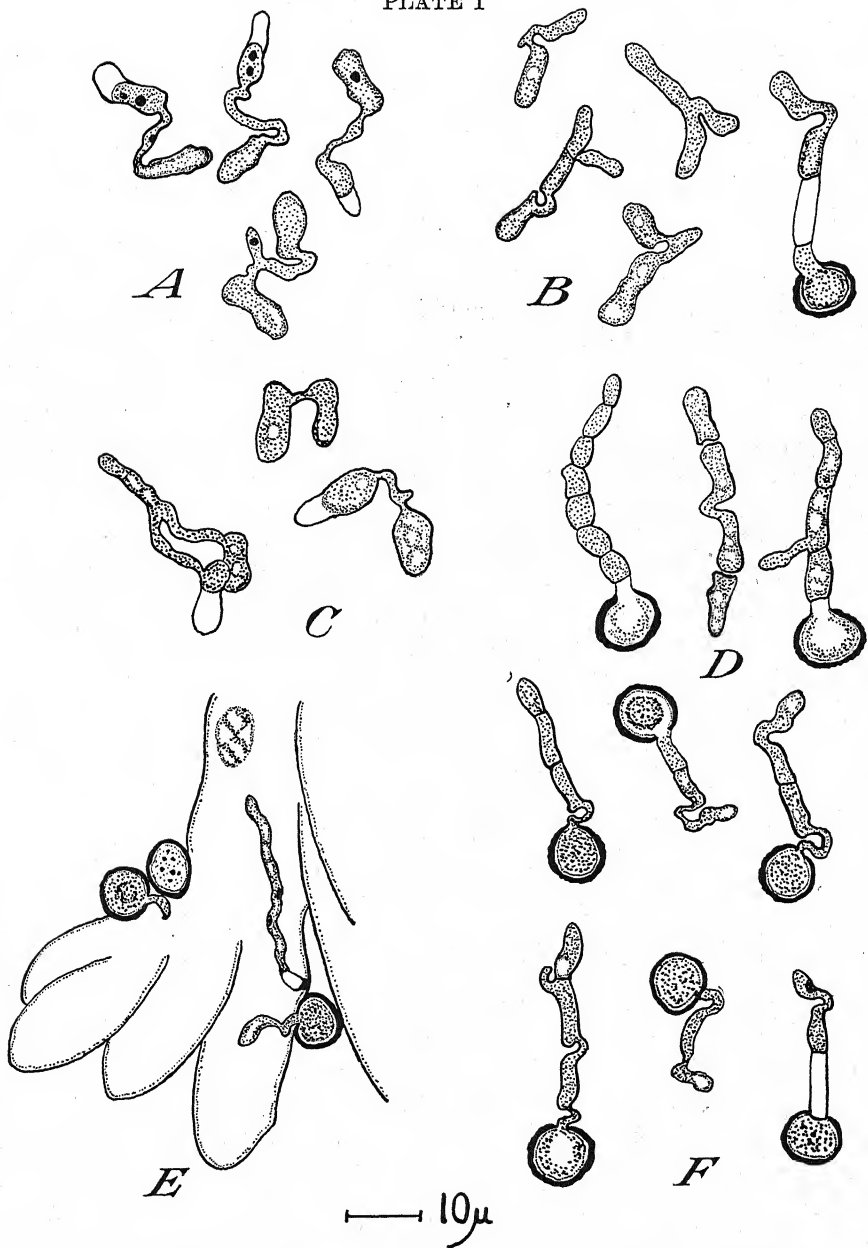
The experiments here considered were carried out in a number of different ways. Oat grains, complete with their enclosing glumes, were inoculated by means of the vacuum pump method described by Allison.<sup>1</sup> Suspensions of (a) chlamydospores and (b) mixtures of two sporidial lines of unlike sex were made in distilled water, 2 per cent malt solution, and a 2 per cent potato dextrose solution. After evacuating the grains, they were allowed to dry for several hours on the bench at room temperature, and were then placed on moist filter paper in Petri dishes and left to germinate at temperatures of 0, 5, 10, 20, 25, and 30 degrees C.

After the young plumules had commenced to grow they were examined, together with the glumes and the external portions of the caryopses, in order to determine the nature of germination of the smut spores and the origin of the infection tubes penetrating the host. For the most part the material was stained with lacto-phenol and cotton blue; but, in particular instances, microtome sections were prepared and differentiated with Bismarck brown, gentian violet, and Gram's iodine. In addition to the mature grains treated in the manner described, a number of oat florets were dusted with chlamydospores, while still on the plant, to provide material for a study of germination under natural conditions. This material was stained with cotton blue,

<sup>1</sup> Allison, C. C. Studies on the genetics of smuts of barley and oats in relation to pathogenicity. Minnesota Agr. Expt. Sta. Tech. Bull. 119. 1936.



PLATE I



A. Fused sporidia from artificial culture. B. Spores germinated within the glumes at 25° C. C. Fused segments of promycelia and sparse production of sporidia. D. Fused sporidia obtained from within the glumes after inoculation with suspension of two monosporidial lines of opposite sex. E. Flower infection of Anthony oats. Germinated spores on the stigmas and ovaries showing fusion of promycelial segments and formation of gemmae. Note the long multiseptate mycelium arising from the spore on the left. F. Spores germinating on the stigma of an Anthony oat. Direct penetration by germ tubes. The infection hypha is exactly similar to those found in seedling infection. Stained with Bismarck brown, gentian violet, Gram's iodine; all others stained with lactophenol cotton blue. F. Spores germinated within the glumes at a temperature of 5° C. Many segments of the promycelia fused but not released.

and a representative sample of young flowers was fixed and cut in wax so that a more detailed examination of the mode of penetration could be undertaken.

#### RESULTS

In these experiments the nature of the nutrient solutions appeared to exert but a slight effect upon the germination of the smut spores. In general, those suspended in distilled water germinated and the promycelia grew almost as quickly as in nutrient solutions.

The effect of temperature was more marked. At 0° C. germination was almost completely inhibited and spores that had been kept for several weeks showed only the initial stages of emergence of the promycelia. In the case of those stored for 6 days at 5° C., germination had taken place and well developed promycelia were present but no sporidia. In many of the promycelia neighboring segments had united by means of a looped fusion tube, but the cells so joined were not separated from the parent structure (Plate I, Fig. F). These fusion tubes appeared between segments at random, and only in exceptional cases were there more than 2 upon any single promycelium.

The spores kept at 10° C. germinated more quickly but failed to produce sporidia. In other respects they were similar to those described above.

There appeared to be very little difference in the nature of germination of the spores incubated at temperatures of 20° and 25° C., and they may, therefore, be conveniently considered together. At these temperatures a few sporidia were produced but they were definitely limited in number. In some cases a detached promycelium showed signs of budding and also the disjunction of segments, thus forming the gemmae frequently observed in the smut fungi (Plate I, Fig. B). Fusion tubes of the type already described were very common, but, at these temperatures, their development was more advanced and the segments had rounded off their ends and frequently broken away from their original position (Plate I, Fig. B). Such detached, but fused, segments resembled very closely in appearance the fused sporidia characteristic of artificial cultures (Plate I, Fig. A). In many such united cells the true infection hypha, or germ tube, could be observed growing out from the loop-shape connection.

When a mixture of 2 monosporidial lines of unlike sex was injected between the glumes of oat seeds and the latter were allowed to germinate at a temperature of 25° C., fusions developed that were indistinguishable from those found in artificial culture (Plate I, Fig. C). A number of young plumules obtained from grains treated in this way were fixed and microtome sections made with a view to determining the manner in which initial penetration of the host took place. The results showed that this was accomplished by means of infection tubes that pierced the epidermal wall in ex-

actly the same manner as those developed from chlamydospores. This process has been described elsewhere (14).

At a temperature of 30° C. no sporidia were formed, germination was definitely poorer, and the promycelia present were frequently contorted. Fusions were not evident.

Anthony, a very susceptible oat, was selected for the study of flower infection. The young stigmas were dusted with dry spores and the florets left on the plant to develop. An examination of ovaries at daily intervals for 6 days showed that spore germination commenced after about 12 hours and normal promycelia developed. A few sporidia were observed, especially in those places where the concentration of spores was high, but no fusions were observed among them. In material inspected on the third day and later, adjoining promycelial segments, not directly in contact with the stigmatic surface, had fused (Plate I, Fig. D), and, occasionally, the promycelium appeared to be developing into a multiseptate mycelium. Actual penetration of the stigmas could easily be seen and in every case it was effected by the promycelium acting as a germ tube and penetrating directly (Plate I, Fig. E). The infection hyphae were similar in all respects to those found in seedling infection (14).

#### DISCUSSION

The actual time and place of the formation of the dikaryophase in the oat-smut fungi and the origin of the cells involved in the fusion process are matters of considerable importance from a genetical point of view.

In a species such as *Ustilago zaeae*, corn smut, the organism is able to live saprophytically in nature, and very extensive budding of conidia takes place. These conidia may disseminate and lodge on corn plants, the younger portions of which are susceptible to attack at any time, and thus the possibility of hybridization and the evolution of new parasitic strains is enormously increased. *U. avenae* is not strictly comparable. Here, a modified form of flower infection is the normal method of entrance of the pathogen into the host. Resting mycelium is formed, either inside the flowering glumes or in the more superficial tissues of the caryopsis, which invades the young seedling on its emergence at the time of germination. The oat plant is susceptible for only a very short time, as, once the leaves have broken through the coleoptile, further infection does not occur. The opportunity for the extensive budding of conidia and their dissemination, therefore, is restricted, partly because spores can reach the flowers only during the short period the glumes are open and, also, for the additional reason that penetration of the young seedling must be effected rapidly. In spite of the fact that sporidia are produced so abundantly in artificial cultures, the present work suggests that under more natural conditions they are very much less numerous and that an alternative type of fusion takes place. This consists of the union

of adjacent promycelial segments, presumably of opposite sex, by means of a fusion tube that ultimately gives rise to the true "infection hypha." These structures have been observed quite frequently in oat-smut fungi, and have been figured, among others, by Brefeld (3), Harper (7), Lutman (11), and Stakman (13). If this type of fusion is mainly responsible for the establishment of the parasitic dikaryophase, obviously the opportunity for hybridization, with the consequent possible changes in pathogenicity, is relatively restricted, since, although a certain degree of recombination is still possible, the two participating nuclei have been derived from a single diploid parent nucleus. A still further modification, in which fusion tubes are completely absent, often occurs when spores germinate on the stigmas or on the surface of the young seedling itself. Under these conditions the promycelium apparently acts as an infection tube and penetrates the host directly. Here again, therefore, the dikaryotic stage, from its commencement consists only of nuclei derived from the single diploid fusion nucleus in the chlamydospore.

These processes may partly explain the remarkable constancy of many physiologic races in nature. It would be too much to assume that such races are genetically pure, or that each consists of but one biotype, but the failure of extensive hybridization may have a considerable bearing on the maintenance of the *status quo*. In all probability most spore collections or biologic species contain spores of slightly differing types, and there are almost certainly multiple factors involved that determine their pathogenicity. There must also be intense competition between such types, and this may determine the apparently constant behavior of the race or form as a whole. On the other hand, the fact that fused sporidia, under certain conditions, can cause an infection of the host plant indistinguishable from that resulting from direct penetration, suggests that the possibility of hybridization, with the consequent production of new parasitic strains, must not be excluded. Their survival would depend upon the breadth of their distribution and their ability to build up a supply of inoculum quickly. This, in turn, would be governed by a whole set of circumstances that probably do not occur together very frequently in nature.

There is still the further possibility that anastomoses or fusions of hyphae within the host may produce new forms of the pathogen. In the writer's opinion this is not probable. The behavior of the oat-smut mycelium within the host has been studied in detail by Kolk (10), Western (14), and others. The mycelium, in a young susceptible seedling, grows towards the growing point and meristems, and the protoplasm tends to become confined to the hyphal tips leaving the portions immediately behind devoid of contents. Each hypha, although it may put out short branches at certain points, remains separate and, as far as the writer's observations go, no anastomosing of mycelia occurs.

In conclusion, it is suggested that the fact that certain physiologic races of *Ustilago avenae* remain as constant as they do may perhaps be accounted for if the more restricted types of sexual fusion discussed here predominate in nature.

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## A BACTERIAL LEAF SPOT OF GERANIUM

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During the late summer of 1934 a leaf spot of *Geranium sanguineum* L., prevalent in the gardens about Ithaca, New York, was called to the attention of the writer. This species of geranium frequently is used as a border plant for beds of perennial ornamentals, and its effectiveness was greatly destroyed by the disease. Many leaves were spotted and brown, while others had died and fallen from the plants.

It was not determined how long the disease had occurred in this locality, but its occurrence did not appear to be an epiphytotic furthered by abnormal weather conditions of that year. The season had been an average one and had shown no extremes of temperature or moisture. A severe case of the disease was observed in the gardens of the Department of Floriculture at Cornell University, where the plants were in an unsheltered place, with shade only from certain perennials about which the geraniums had been planted. Also the disease was equally severe in a planting in the garden of Professor H. H. Wetzel, where the plants were almost completely shaded by trees. The trouble again was evident in 1935, but possibly not in so severe a form. The heavy rains of July of this year seemed to have had little effect upon infection.

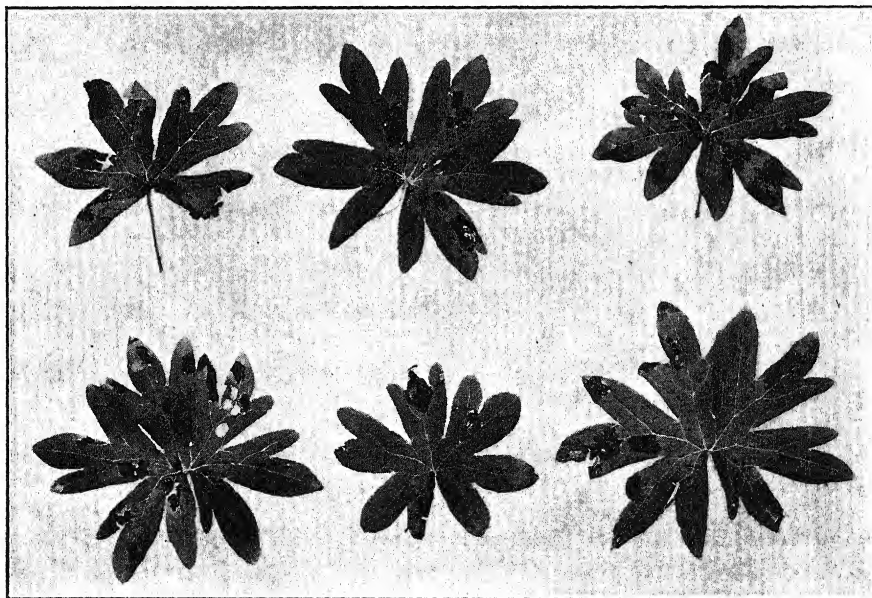


FIG. 1. Bacterial leaf spot of *Geranium sanguineum* caused by *Phytomonas geranii*.

The symptoms of the disease on the geranium are as follows: The spots appear primarily on the leaves, and are not large, approximately 25 mm. in diameter, on the average; but a number of adjacent spots may anastomose and cover larger areas on the leaves. (Fig. 1.) On the upper side of the leaf the lesions are dark brown, with frequently a reddish tinge. On the under side a slight water-soaked condition may be observed, which is characteristic of many of the bacterial leaf spots. The spots are confined to the spaces between the larger veins on the leaves, but, nevertheless, they are seldom angular in shape. As a rule very little effect is observed on the noninfected tissue, but frequently several spots may cut off an entire lobe of a leaf and the latter will curl up, turn brown, and die. Less frequently are the spots so numerous that the entire leaf dies, but when this does occur it is on the young leaves only. In certain cases leaves with a number of lesions on them have shown a slight yellowing of the noninfected tissue, and at times a deep reddening of this tissue.

Only a few lesions have been observed on the stems or petioles of the geranium and these appeared to have occurred on young tender parts.

A microscopic examination of a smear<sup>1</sup> from a lesion on a leaf will show a large number of bacteria in the tissue. The organisms have not been observed in the vascular system. In the plant the bacteria are medium-size rods and usually occur singly. Dilution plates with beef-extract-peptone agar as a medium have been made from a number of lesions. In 24 to 48 hours all plates have yielded colonies of a yellow pigmented bacterium, very similar in appearance to *Phytomonas campestris* or *Phyt. phaesoli*.

#### PATHOGENICITY

No healthy specimens of *Geranium sanguineum* were available for inoculation experiments in the late summer of 1934. Therefore, the pathogenicity of the isolates was not tested until the following year. By that time roots of the geranium had been planted in the greenhouse in pots and healthy plants produced. Further isolations of the yellow organism were made from diseased plants to provide young cultures for inoculation experiments. The experiments were conducted in the following manner. The healthy plants were kept in a greenhouse at a temperature of approximately 75° F. The

<sup>1</sup> To make a microscopic examination for bacteria in lesions on plants, the following method has been used for some years with satisfactory results. A small portion of the lesions is placed on a clean slide in a drop of a 2 per cent aqueous solution of congo red. The tissue is chopped up finely in the stain with a scalpel, is allowed to macerate a minute, and then carefully scraped off the slide. The remaining stain is spread in a thin film over the slide and allowed to dry without applying heat. A few drops of acid alcohol are run over the film to change the color of the dye to a deep blue, which is more distinct under the microscope. The smear is then examined with the oil emersion lens without a cover glass. The oil is placed directly upon the stain. The bacteria stand out as unstained particles against a dark background. This method is rapid and usually successful, unless the plant juices coagulate the stain.

bacteria were grown on beef-extract-peptone agar slants in a 27° C. incubator, and when used were from 24 to 48 hours old. They were applied by painting with a camel-hair brush a water suspension of the organism over the geranium leaves. The leaves of certain plants were pricked with insect needles before inoculation, while others were left uninjured. After inoculation, certain of the plants were placed under bell glasses; others were left on the benches in the greenhouse under prevailing conditions. Thus, various environmental conditions were produced.

Infection was obtained in all cases. The best infections occurred when the leaves were injured previous to inoculation. At least, more spots appeared with greater certainty and, with this technique, the incubation period was somewhat shorter than when the leaves were left uninjured. Spots appeared within 1 to 2 weeks and were at first more evident on the under side of the leaf.

Although injury to the leaves is a more certain and a quicker method of obtaining infection, stomatal invasion, no doubt, occurs. Infection was obtained on uninjured leaves a sufficient number of times to lead one to believe that it occurred not merely through chance injuries, but also through stomates. Furthermore, when the disease was once established in a plant, the young leaves frequently became infected shortly after they unfolded. The older leaves appeared more resistant.

The causal organism was reisolated in great numbers from artificially produced lesions.

Some attempts were made to determine the host range of this organism after its pathogenicity was established on *Geranium sanguineum*. In the greenhouse, inoculation experiments were conducted on growing plants of *G. maculatum* L. These were made by slightly pricking the leaves with insect needles and then applying a water suspension of bacteria with a camel-hair brush. Infection was obtained on the young leaves, but not on the older, mature leaves. The lesions were similar to those on *G. sanguineum*, but possibly not so large. *Geranium maculatum* appears to be somewhat resistant to the disease. Observations were made on several plantings of this species in gardens about Ithaca, New York, and on the species growing wild in this locality, but the disease was not found.

Further observations and work pertaining to the host range of the pathogen in the genus *Geranium* are as follows: Several plants of *G. pratense* L. and *G. sylvaticum* L. were growing in the gardens of the Department of Floriculture at Cornell in the same bed with the diseased *G. sanguineum*. Examination of their leaves in the late summer of 1935 revealed small necrotic spots similar to those under discussion in this article. Microscopic examination, however, showed only a few bacteria, and attempts to isolate a pathogen were unsuccessful. In the spring of 1936 the young shoots of these two species became severely diseased. The spots on the leaves



became much larger than those on *G. sanguineum* and many of the stems turned brown and died. Isolations from lesions on both *G. pratense* and *G. sylvaticum* yielded bacteria similar in appearance to the geranium pathogen under discussion. The isolate from *G. pratense* caused good infection on leaves of *G. sanguineum* in the greenhouse. This also was true of the isolate from *G. sylvaticum*. Due to the fact, however, that healthy plants of these two species were not available at the time, reciprocal inoculation experiments could not be made with isolates from *G. sanguineum*. Nevertheless, from the appearance of the bacteria from the three species of Geranium, and from the appearance of the lesions they cause on *G. sanguineum*, there seems to be little doubt that they are identical.

Inoculation experiments also were made on species of plants outside the genus Geranium. Twice, attempts were made to infect the common house geranium, *Pelargonium hortorum* Bailey. The plants were of the common red-flowering variety and the experimental procedure was the same as that described above for *G. sanguineum*. Some plants were left uninjured, while others were pricked with insect needles before inoculations. In no case did infection take place, although *G. sanguineum* was inoculated at the same time and became infected. The red Pelargonium appears not to be susceptible.

Due to the fact that the geranium organism, when grown on beef-extract agar and on potato-dextrose agar, appeared identical with *Phytomonas campestris* and *Phyt. phaseoli*, cross inoculations were made on young cauliflower plants (*Brassica oleracea* var. *botrytis* L.) and on young pods of *Phaseolus vulgaris* L. Negative results were obtained in all trials. Mr. C. Wernham is conducting further cross-inoculation experiments with this pathogen in a study of a group of similar organisms. His work will be reported sometime in the future.

The pathogen may overwinter in the lesions on the leaves of *Geranium sanguineum* and possibly other species of this genus. This was demonstrated in the following manner: Plants that had wintered under mulch and snow during the season 1935-36 were examined for leaves late in March. A few leaves were found, still alive and rather bronze in color, but exhibiting small necrotic lesions typical of the disease. Dilution plates were made from these lesions and a yellow bacterium was isolated that was identical in appearance with the pathogen. Two isolates from such material were saved and tested for pathogenicity in the greenhouse on healthy plants of *G. sanguineum*. Both proved to be virulent cultures of the pathogen.

#### THE CAUSAL ORGANISM.

The pathogen causing the leaf spot of geranium is readily isolated from the lesions on the leaves of the host and grows well on both beef-extract-peptone agar and on potato-dextrose agar. A number of isolates have been in culture and 3 of these were used in describing the bacterium. Two were

isolated in late May of 1935 from 2 different plants and the third was a reiso-late of one of these, made in June, 1935. All 3 behaved similarly in culture. A description of the organism follows.

*Morphology.* The pathogen is a mid-size rod with rounded ends, occur-ring singly, in pairs, or occasionally in short chains. Cultures in beef-ex-tract-peptone agar (pH 6.8), incubated at 27° C. for 24 hours, showed the following dimensions for the cells:  $2\ \mu$  (1.05 to  $3.15\ \mu$ ) by  $.75\ \mu$  ( $.4$  to  $1.05\ \mu$ ). Congo red negative stains were used in these measurements.

The organism is motile by a single polar flagellum, which is approximately twice the length of the bacterium. The pathogen is Gram-negative.

*Cultural Characteristics.* On beef-extract-peptone agar slants (pH 6.8 to 7.0) at 27° C. a moderate growth, filiform, glistening and primuline yellow, develops along the streak in 24 hours. The edges are entire. The consis-tency of the growth is watery to butyrous. On potato-dextrose agar slants, the growth is more abundant, gummy in appearance and a lighter yellow. In beef-extract bouillon (pH 6.8) a cloudy growth appears in 24 hours. No pellicle is formed but there is a good sediment. In Clara's medium<sup>2</sup> a very slight clouding appears, but no pigment is produced. In brom-creosol-purple milk at 27° C., a slight surface clearing is observed in 48 hours, which gradually extends downward until after 3 weeks the medium is a clear purple liquid with a muddy sediment. A few crystals have been observed in the sediment, probably tyrosine, but no tests have been made for them. In shake cultures with beef-extract-peptone agar, plus .5 per cent dextrose used as a medium, colonies appeared only 1 mm. below the surface, showing that the pathogen is an aerobe.

*Biochemical reactions.* A study of the biochemical reactions of the pathogen in culture was made and is reported here.

Growth in a gelatin stab culture is good and liquefaction begins in about two days; at first turnip-shape, it progresses at a moderate rate. A light cloudy growth appears after 48 hours in the synthetic nitrate medium listed in the Manual of Methods.<sup>3A</sup> Tests for nitrites with sulfonilic acid and a-naphthalamine in acetic acid give a negative reaction, but tests for am-moniam are positive in the cultures and negative in the check medium. It ap-pears from the growth of the bacteria and from the presence of ammonia in the synthetic medium that some of the nitrates are utilized, but if reduced to nitrites, these are utilized immediately. The pathogen when grown in beef-extract-peptone bouillon produces ammonia. Tests were made accord-ing to Hansen's method.<sup>3B</sup> In determining whether or not the bacteria pro-duce hydrogen sulphide, they were grown in Bacto-triptyophane broth and

<sup>2</sup> Clara, F. M. A comparative study of the green-fluorescent bacterial plant pathogens. New York (Cornell) Agr. Exp. Sta. Mem. 159. 1934.

<sup>3</sup> Society of American bacteriologists, Committee on bacteriological technique. Manual of methods for the pure culture study of bacteria. The Society, Geneva, N. Y. 1923 to date. [Looseleaf.] A. Leaflet II, Ed. 6, 1936, p. 15. B. Leaflet VI, Ed. 6, 1935, p. 12. C. Leaflet V, Ed. 5, 1934, p. 19.

strips of filter paper, impregnated with lead acetate, were hung from the plugs, as recommended by Zobell.<sup>3c</sup> A light brown coloring of the filter paper showed that a small amount of  $H_2S$  had been produced. The organism, also, grown in the same broth, was tested for indol production on the 1st, 2nd, and 7th days. The Ehrlich-Böhme test was used, and was negative in all cases.

Fermentation studies of the organism were made in the following manner: An inorganic synthetic medium was used to which 1 per cent carbohydrate was added, or, in the case of the salts of the organic acids, .15 per cent was added. The synthetic medium was that recommended in the Manual of Methods.<sup>3A</sup> As a rule the media were sterilized by autoclaving at 15 pounds' pressure for 18 minutes. In the following instances, where a sugar was liable to break down or to carbonize, a 10 per cent solution of the sugar was filtered through a Berkefeld filter (N.). A sufficient amount of this sugar was then added to the inorganic medium, which had been heat-sterilized in tubes, to make a concentration of 1 per cent carbohydrate. These sugars, which were filtered, were levulose, arabinose, xylose, lactose, maltose, sucrose, and raffinose.

A different method from that described above was used in the study of the fermentation of starch and of cellulose. For the starch, the customary starch agar was employed with the iodine test. For the cellulose test, strips of filter paper were placed in tubes and a small amount of a carbohydrate-free synthetic medium was added.

In these studies the 3 isolates were able to utilize the following carbon sources: dextrose, galactose, levulose, xylose, rhamnose, lactose, maltose, sucrose, raffinose, glycerol and the sodium salts of citric, malic, malonic, lactic, and succinic acid. Arabinose, starch, cellulose and the sodium salts of formic, hippuric, maleic, and tartaric acid were not fermented. There was some doubt as to the fermentation of mannitol, salicin, and sodium acetate.

#### TAXONOMY

No bacterial plant pathogen, so far as the writer is aware, has been described as causing a leaf spot on a member of the genus *Geranium*. Several have been reported on nearby genera, however. Lewis<sup>4</sup> described a green fluorescent bacterium which caused a leaf spot of species of *Erodium* and *Pelargonium*, and later Miss Brown<sup>5</sup> reported a similar species, *Phytomonas pelargoni* on the latter host. Miss Brown also mentions bacterial diseases of *Pelargonium* reported from Massachusetts and minor references made by E. F. Smith to a similar trouble.

*Phytomonas erodii*, described by Lewis, belongs to the green fluorescent group of bacteria, and *Phyt. pelargoni* is a similar species, but does not pro-

<sup>4</sup> Lewis, I. M. A bacterial disease of *Erodium* and *Pelargonium*. *Phytopath.* 4: 221-232. 1914.

<sup>5</sup> Brown, Nellie A. Bacterial leafspot of *Geranium* in the eastern United States. *Jour. Agr. Res. [U. S.]* 23: 361-372. 1923.

duce this pigment. Passalacqua<sup>6</sup> reports a green fluorescent bacterium causing a disease of Pelargonium in Italy, but does not give it a name. The pathogen on Geranium is distinct from the above reported species in appearance and as far as they are described, distinct in many of its cultural characteristics. The organism pathogenic to Pelargonium, with which E. F. Smith and J. R. Johnston worked, was yellow, according to Miss Brown,<sup>5</sup> and motile by a single flagellum. These two characters fit the geranium organism, but only a guess could be made on the relationship of the two, since nothing further is known concerning the Smith pathogen. Furthermore, the geranium leaf-spot bacterium does not appear to be pathogenic on Pelargonium. In view of these facts the writer believes he is working with an undescribed species of bacterium and, therefore, the following name is proposed: *Phytomonas geranii*, sp. n.

#### BRIEF DESCRIPTION

*Phytomonas geranii* is a mid-size rod with rounded ends, occurring singly, in pairs, or occasionally in short chains. The average size is  $2\mu$  by  $.75\mu$ . It is motile by 1 polar flagellum and is Gram-negative. The pathogen is an aerobe.

On agar slants growth is good, filiform, and yellow. The consistency of the culture is watery to butyrous; bouillon cultures are cloudy in 24 hours; gelatin is liquefied; milk becomes alkaline and is cleared; nitrates are reduced; ammonia is formed, as is also  $H_2S$ ; indol is not produced. The following carbon sources are utilized: dextrose, levulose, galactose, xylose, rhamnose, lactose, maltose, sucrose, raffinose, glycerol; and the sodium salts of citric, lactic, malic, malonic, and succinic acid. The following carbohydrates were tested and were not fermented; arabinose, starch, cellulose; and the sodium salts of formic, hippuric, maleic, tartaric, and salicylic acids.

The organism is pathogenic on *Geranium sanguineum* L. and *G. maculatum* L., *G. pratense* L. and *G. sylvaticum* L.

#### SUMMARY

A bacterial leaf spot of Geranium prevalent about Ithaca, New York, appears to be new to literature and a description is given of its symptoms. The causal organism was isolated, its pathogenicity proved and an extensive description is given of the pathogen. The name *Phytomonas geranii*, sp. n., is proposed. The organism is pathogenic on *Geranium sanguineum*, *G. maculatum*, *G. pratense*, and *G. sylvaticum*. Infection was not obtained on *Pelargonium hortorum*. The pathogen was found to overwinter in the lesions on the leaves of *G. sanguineum*.

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<sup>6</sup> Passalacqua, T. La variegatura patologica del Pelargonium ed altre piante. R. Ist. Bot. Palermo Lav. 4: 201-240. 1933. [Abstract in Rev. App. Mycol. 13: 444. 1934.]

# CLASSIFICATION OF LILY-MOSAIC VIRUS<sup>1</sup>

W. C. PRICE

(Accepted for publication January 28, 1937)

## INTRODUCTION

The Easter lily, *Lilium longiflorum* Thunb., is known to be attacked by 3 separate and distinct virus diseases. These are yellow flat (5), lily mosaic (1), and celery mosaic<sup>2</sup> (13). Yellow flat is readily distinguished from the other 2 diseases by its failure to produce striping or mottling of the foliage. Celery mosaic is now known to be caused by a virus belonging in the cucumber-mosaic virus group (9). The symptoms of celery mosaic in lily as reported by Wellman (13) closely resemble those described for the classic lily-mosaic disease. Because of this resemblance and because both lily mosaic and celery mosaic are transmitted by *Aphis gossypii* Glover (2, 11), it seemed likely that the causal agents of the 2 diseases might be related, if not identical. A study was, therefore, undertaken to determine whether or not lily-mosaic virus should be classified in the cucumber-mosaic virus group. The results of the study indicate that it is closely related to cucumber-mosaic virus and should, therefore, be placed in the cucumber-mosaic virus group. It is the purpose of this paper to present the data on which these conclusions are based.

## REVIEW OF LITERATURE

Probably the earliest description of what is now known to be lily mosaic was that by Stewart (10) in 1895. There is, however, ample reason to believe that the disease was prevalent in lilies in various parts of the world some years previous to the publication of this description. It was probably introduced into Bermuda about 1893 (14) where, in association with the yellow flat disease, it caused a rapid decline of the lily industry of the island. At that early date nothing was known about the etiology of the disease. Indeed, it was not until 1928, when Guterman (1) reported mechanical transmission of the mosaic disease of *Lilium auratum* Lindl., that it was proved to be caused by an infectious agent belonging in the virus group. In 1930 Guterman (2) reported that mosaic of Easter lily could be transmitted either by mechanical inoculation or by means of *Aphis gossypii* and that it occurred

<sup>1</sup> Published at the expense of The Rockefeller Institute for Medical Research, Princeton, N. J., out of the order determined by the date of receipt of the manuscript. This practice in nowise delays the publication of manuscripts printed at the expense of The American Phytopathological Society or other agency.

<sup>2</sup> Wellman (13) reported that, in addition to celery mosaic, the lily is attacked by at least one other virus disease in Florida, but he did not state whether this is lily mosaic, yellow flat, or some other virus disease.

in many different species of lily, as well as in the genus *Fritillaria*. Both Guterman (2) and Ogilvie and Guterman (6) recognized 3 different symptom pictures, but Guterman (2) regarded these as different manifestations of the same disease rather than as different diseases.

#### MATERIALS AND METHODS

The lily-mosaic virus used for most of the studies to be reported was obtained from a stock of diseased bulbs of "*Lilium giganteum*" purchased in May, 1935, from a commercial grower. Some of the experiments with this virus were repeated with a virus secured from Mr. D. K. O'Leary of the Boyce Thompson Institute for Plant Research, Yonkers, N. Y. Virus-free plants of *L. longiflorum* were likewise obtained from Mr. O'Leary. These were either grown from seed or were from selected cage-grown stock.

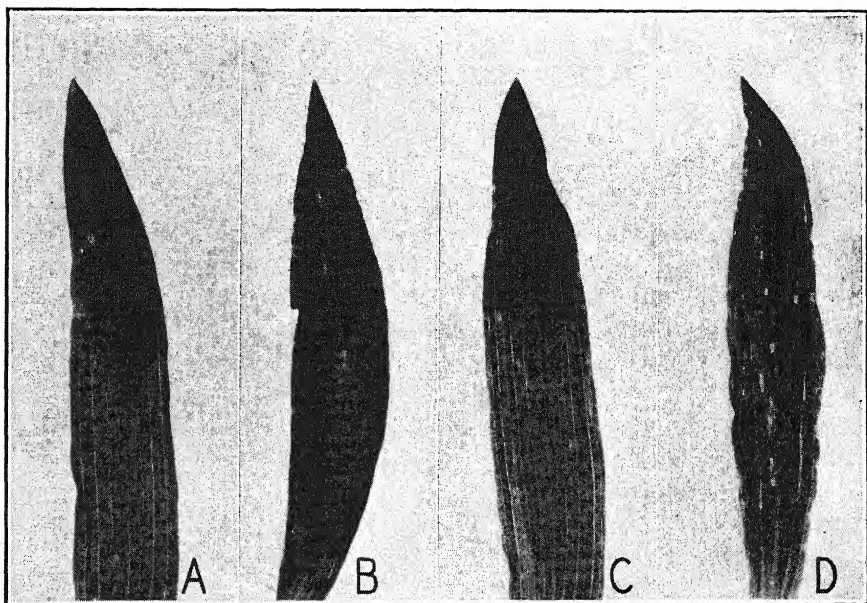
The cucumber- and celery-mosaic viruses used were the same as those employed in previous work by the writer (7, 9).

Plants were grown in 4-inch or 6-inch porous clay pots in a greenhouse that was fumigated frequently to keep down insects. No aphids were observed on any of the plants used in the experiments to be reported.

Inoculations were made by means of the rubbing method. In some instances, carborundum was used as an abrasive, but the addition of this material did not appear to increase the percentage of infection in any of the experiments to be reported.

#### TRANSMISSION OF STRAINS OF CUCUMBER-MOSAIC VIRUS TO *LILIUM LONGIFLORUM*

A number of attempts to transmit cucumber mosaic to lily were made, but not all of these were successful. In about half of the experiments a low percentage of infection was obtained in *Lilium longiflorum* with cucumber-mosaic virus, cucumber-mosaic strain 6 virus, and celery-mosaic virus. The symptoms appeared 10 days after inoculation and were similar to those produced by lily-mosaic virus in the same host. They consisted of elongated yellow spots, which gradually enlarged and in some instances coalesced to produce a mosaic-like mottling of affected leaves. In many cases, the centers of the spots became water-soaked, dried out, and turned brown, producing a typical necrotic spotting similar to that described previously for lily mosaic in *L. longiflorum* (6, 2). The necrotic spotting is illustrated in figure 1. The symptoms of the 3 strains of cucumber mosaic and those of lily mosaic in *L. longiflorum* were so much alike that it was impossible to differentiate the viruses on this basis alone. There was, however, considerable variation in the symptoms shown by plants infected with each of the viruses. Several failed to develop the necrotic spotting symptom and showed only a mild mottling. All of the plants were stunted, but some were more severely stunted than others.



Photograph by J. A. Carlile

FIG. 1. Leaves of *Lilium longiflorum* showing mottling and necrotic symptoms produced by different viruses. A. Lily-mosaic virus. B. Cucumber-mosaic virus. C. Cucumber-mosaic strain 6 virus. D. Celery-mosaic virus.

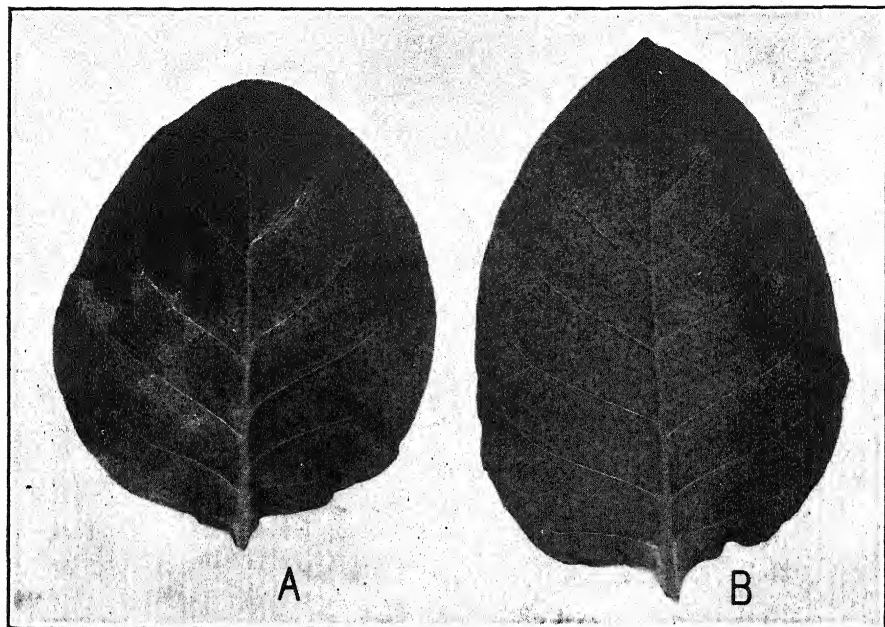
The viruses of cucumber mosaic, cucumber-mosaic strain 6, and celery mosaic were transferred from diseased lilies to healthy Turkish tobacco and each produced its typical symptoms in this host.

#### TRANSMISSION OF LILY MOSAIC TO TOBACCO

A number of attempts to transmit mosaic from *Lilium longiflorum* to healthy Turkish tobacco, *Nicotiana tabacum* L., were made. Negative results were obtained in about one half of the attempts and positive results in the remainder. The first symptoms to appear were primary lesions consisting of zonate yellow spots with or without a necrotic periphery (Fig. 2). The primary lesions were observed in from 3 to 8 days after inoculation. Systemic symptoms were either absent or consisted of only a few discrete zonate yellow or necrotic lesions or of yellowed areas surrounded by zigzag necrotic lines.

When first isolated from lily, the virus of lily mosaic remained localized in tobacco or produced only a few scattered systemic lesions. After several passages in tobacco, using the inoculated leaf as a source of inoculum in each instance, some of the infected tobacco plants developed systemic mottling symptoms. Isolation from the mottled leaves yielded a virus that moved





Photograph by J. A. Carlile

FIG. 2. A. Primary lesions produced in Turkish tobacco by lily-mosaic virus from *Lilium longiflorum*. B. Healthy Turkish tobacco leaf for comparison.

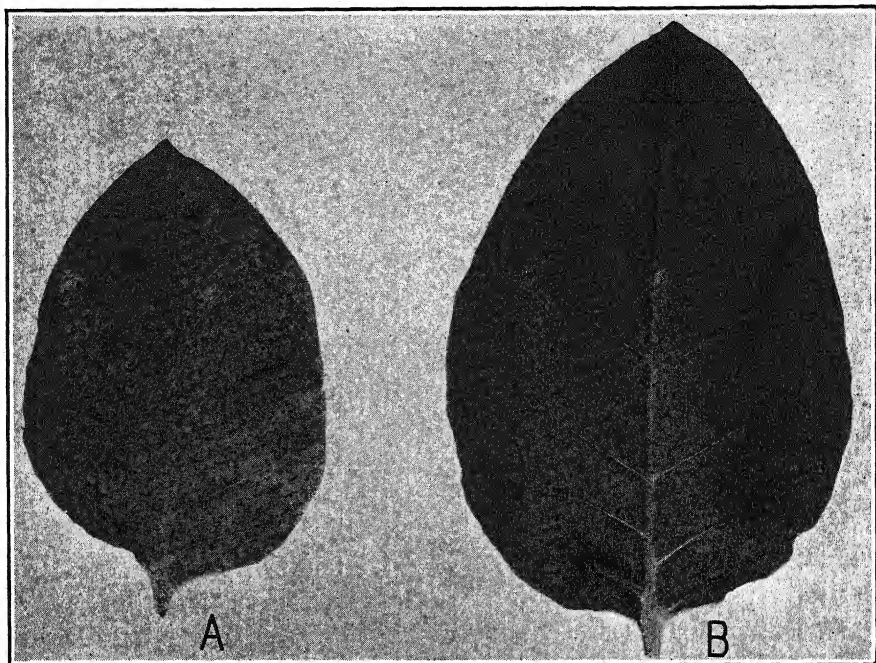
readily and produced distinctive mottling symptoms. It is believed that this virus arose from the lily-mosaic virus and that it was selected out by passage. It will be referred to as the passage strain of lily-mosaic virus.<sup>3</sup> Transfer of this passage strain of lily-mosaic virus to tobacco elicits primary lesions of the yellow rather than of the necrotic type. It moves rapidly into the young leaves and produces there a characteristic mottling disease (Fig. 3). When transferred back to *Lilium longiflorum*, it produces symptoms identical with those of lily mosaic.

#### TRANSMISSION OF LILY MOSAIC TO CUCUMBER

The passage strain of lily-mosaic virus was readily transferred from tobacco to the Improved Long Green and Early Fortune varieties of cucumber, *Cucumis sativus* L. Infected plants showed a bright yellow chlorosis along the veins (Fig. 4), and in some instances a mild yellow mottling. The virus was transferred from infected cucumber back to tobacco.

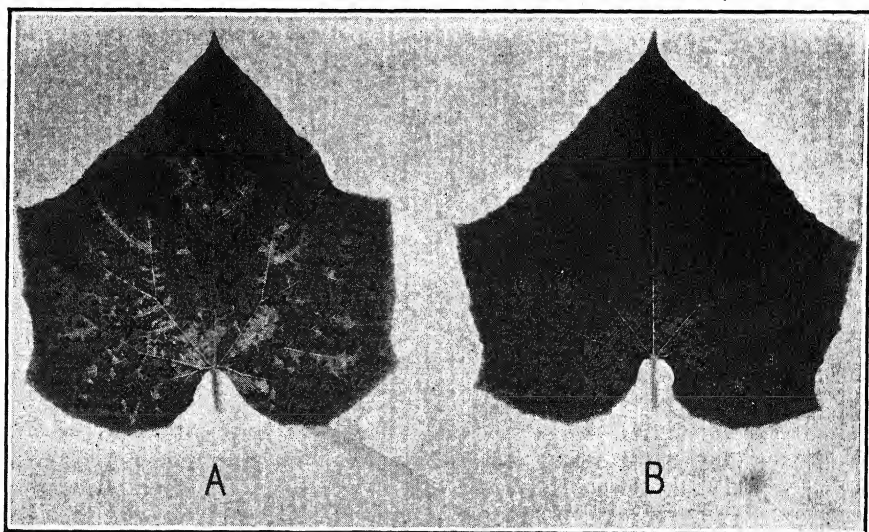
<sup>3</sup> On a number of different occasions rapid-moving strains have been isolated from the lily-mosaic virus secured from Mr. O'Leary, as well as from that obtained through a commercial grower. The strains isolated on these different occasions may be distinct or they may be identical. If distinct, then they differ from one another only in minor particulars. To avoid confusion in reporting the results of this study, they have been grouped and considered as identical.





*Photograph by J. A. Carlile*

FIG. 3. A. Systemic mottling symptoms produced in Turkish tobacco by passage strain of lily-mosaic virus. B. Leaf of healthy Turkish tobacco for comparison.



*Photograph by J. A. Carlile*

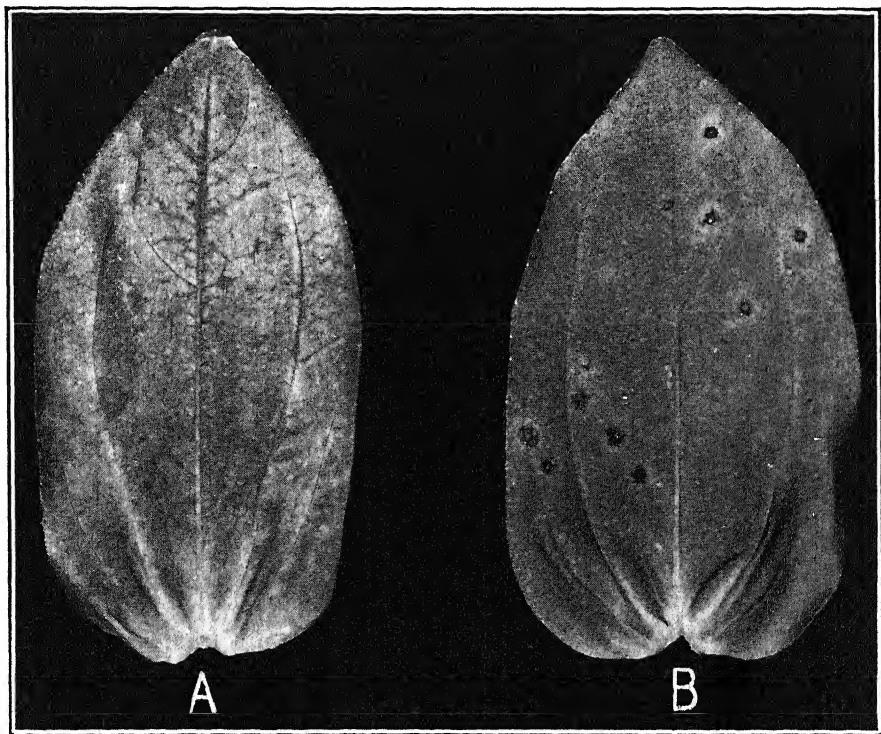
FIG. 4. A. Systemic chlorosis produced in a leaf of Improved Long Green cucumber by passage strain of lily-mosaic virus. B. A healthy cucumber leaf for comparison.

## IMMUNOLOGICAL STUDIES

Virus was transferred directly from diseased lily plants to healthy zinnia, *Zinnia elegans* Jacq. var. Golden Gem Midget, plants. It produced yellow primary lesions but did not become systemic in any of the infected plants.

The passage strain of lily-mosaic virus was transmitted to healthy zinnia plants. It produced systemic symptoms consisting of yellow and green mottling similar to the mottling produced by certain strains of cucumber-mosaic virus in zinnia. It was transmitted from zinnia back to tobacco in which it produced typical mottling symptoms.

The writer (8) has shown that leaves of zinnia thoroughly invaded by mottling strains of cucumber-mosaic virus are immune from virus of cucumber-mosaic strain 6, which produces necrotic lesions in healthy zinnia



Photograph by J. A. Carlile

FIG. 5. Zinnia leaves photographed 6 days after inoculation with cucumber-mosaic strain 6 virus. A. This leaf was infected by the passage strain of lily-mosaic virus and showed mottling symptoms at the time of inoculation with virus of strain 6. It did not develop lesions. B. This leaf was healthy at the time of inoculation with virus of cucumber-mosaic strain 6. It shows necrotic lesions produced by the latter virus.

plants, and, secondly, that infection of zinnia with viruses unrelated to that of cucumber mosaic does not protect them against infection with strain 6 virus. This technique was used in the present study to determine the relationship between cucumber-mosaic and lily-mosaic viruses.

Ten young zinnia plants were inoculated with the passage strain of lily-mosaic virus. Ten similar plants were kept uninoculated for controls. All 10 of the inoculated plants became infected and developed mottling symptoms. After 19 days, 6 mottled leaves on each of the diseased plants and 6 leaves on each of the healthy control plants were thoroughly rubbed with virus of cucumber-mosaic strain 6. A total of 470 necrotic lesions developed in the 60 control leaves, but not a single lesion appeared in the leaves infected with lily-mosaic virus. The results are illustrated in figure 5. The experiment was repeated with an additional lot of 20 zinnia plants, using again the passage strain of lily-mosaic virus, and similar results were obtained.

The data show that leaves of zinnia plants thoroughly mottled by the passage strain of lily-mosaic virus are immune from infection with virus of cucumber-mosaic strain 6, and thus indicate that the 2 viruses are closely related. It is concluded that lily-mosaic virus should be classified in the cucumber-mosaic virus group.

#### DISCUSSION

It is well known that slow-moving strains of tobacco- and cucumber-mosaic viruses in tobacco mutate and give rise to strains that move more rapidly in this host (3, 7). These rapid-moving strains may be isolated from the slow-moving strains by inoculation from the young leaves of tobacco plants infected with the latter. It seems evident that the passage strain of lily-mosaic virus was produced from the ordinary strain by a similar process. The evidence that lily-mosaic virus is related to cucumber-mosaic virus is no less conclusive because it is indirect. By means of an immunological technique, it has been shown that the passage strain of lily-mosaic virus belongs in the cucumber-mosaic virus group. It follows that ordinary lily-mosaic virus, from which the passage strain was derived, likewise belongs in the cucumber-mosaic virus group.

Demonstration of the relationship of lily-mosaic and cucumber-mosaic viruses adds an additional virus strain to the already diverse cucumber-mosaic virus group. It seems likely that further study will show many of our common virus diseases to be caused by viruses belonging in this same group. For instance, McWhorter (4) has reported that tulip mosaic or "breaking" is caused by the interaction of 2 viruses, one of which carries a color-removing factor and produces striping of the leaves. He found that the leaves of mosaic-diseased *speciosum* lily carry a virus similar to or identical with the color-removing virus of tulip mosaic. If these viruses are

indeed identical, then the color-removing component of the tulip-mosaic virus complex may belong in the cucumber-mosaic virus group. As another example, Wellman (12, 13) has shown that the virus of southern celery mosaic when transferred to banana, *Musa sapientum* L., causes a disease similar to bunchy top of banana. This result suggests that the virus of bunchy top may be related to or identical with the virus of celery mosaic and may also belong in the cucumber-mosaic virus group.

#### SUMMARY

The symptoms of cucumber-mosaic, cucumber-mosaic strain 6, and celery-mosaic viruses in *Lilium longiflorum* Thunb. were found to be similar to those of lily-mosaic virus in this host.

When transferred from *L. longiflorum* to Turkish tobacco, *Nicotiana tabacum* L., lily-mosaic virus caused primary necrotic lesions and remained localized, producing only an occasional systemic lesion. On passage from tobacco to tobacco, lily-mosaic virus gave rise to a strain that became systemic and produced mottling symptoms.

The passage strain of lily-mosaic virus was transferred to cucumber, *Cucumis sativus* L., and to zinnia, *Zinnia elegans* Jacq. Leaves of zinnia plants thoroughly invaded by the passage strain of lily-mosaic virus were found to be immune from infection with virus of cucumber-mosaic strain 6. It is concluded that lily-mosaic virus should be classified in the cucumber-mosaic virus group.

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## THE COMPARATIVE RÔLE OF CERTAIN NEMATODES AND FUNGI IN THE ETIOLOGY OF DAMPING OFF, OR SORESHIN, OF COTTON

C. H. ARNDT AND J. R. CHRISTIE

(Accepted for publication November 19, 1936)

In a former paper<sup>1</sup> reference was made to investigations regarding the rôle of nematodes in the etiology of lesions on the hypocotyls of cotton seedlings, and it was stated therein that complete results would be published later. The results of these experiments, insofar as they pertain to nematodes, were, for the most part, negative, and a greatly detailed report appears to be unnecessary.

The experiments were conducted in a greenhouse of the South Carolina Experiment Station at Clemson, S. C. Both nematodes and fungi were reared on culture media. The soil was steamed for 2 hours, cooled, the inoculum then mixed with the upper portion of the soil, and the cotton seed planted. The seed was acid-delinted and in all cases surface-sterilized by immersion for 3 minutes in  $\frac{1}{2}$  per cent  $\text{HgCl}_2$  in 50 per cent ethyl alcohol. The water content of the soil was previously adjusted to and then maintained at 60 per cent of its water-holding capacity.

In the experiments conducted during November, 1933, 48 sheet-metal cylinders or cans (25 × 30 cm.) were used, each accommodating 21 seedlings. The cans were divided into 4 sets and the soil maintained at 18, 21, 25 and 30 degrees C., respectively. The cans of each set were divided into 3 lots; one lot was infested with *Aphelenchoides parietinus* (Bastian, 1865), one lot with *Aphelenchus avenae* Bastian, 1865, and the third lot was left uninfested, to serve as control.

<sup>1</sup> Christie, J. R., and C. H. Arndt. Feeding habits of the nematodes *Aphelenchoides parietinus* and *Aphelenchus avenae*. *Phytopath.* 26: 698-701. 1936.

In the experiments conducted in February, and March, 1935, 160 8-liter, stoneware crocks were used, each accommodating 18 seedlings. The crocks were divided into 20 lots of 8 each. Four species of nematodes, *Aphelenchoides parietinus*, *Aphelenchus avenae*, *Cephalobus elongatus* de Man, 1880, and *Acrobeles bütschlii* (de Man, 1885), and 3 species of fungi, *Fusarium moniliforme* Sheld., *F. vasinfectum* Atk., and *Glomerella gossypii* Edgerton were used. One set of crocks served for noninfested controls. One set each was used for the 4 species of nematodes, 1 set each for the 3 species of fungi, and 1 each for a species of nematode in combination with a species of fungus, all 12 possible combinations being employed.

In the experiments conducted in January, and February, 1936, the 4 species of nematodes used in the preceding experiments were combined to form mixed cultures and are here referred to as "nematodes." Forty-eight cans were used, each accommodating 21 seedlings. The cans were divided into 3 sets of 16 each and the soil maintained at 21, 24, and 27 degrees C., respectively. The sets were divided into 8 lots of 2 cans each and 1 set was devoted to each of the following: Noninfested controls, nematodes only, *Fusarium moniliforme* only, *F. vasinfectum* only, *Glomerella gossypii* only, nematodes + *F. moniliforme*, nematodes + *F. vasinfectum*, and nematodes + *G. gossypii*.

In every experiment the technic employed appeared to be satisfactory. In all cases the nematode-inoculated soil harbored a heavier infestation of these organisms than would ordinarily be found in nature. Almost without exception the lesions that developed on seedlings growing in nematode-inoculated soil were infested with nematodes, usually in large numbers. In no case was any species of nematode found other than the one used in inoculating the soil. Nematodes were never found in the controls or where the soil was inoculated with a fungus only.

The experiments failed to furnish convincing evidence that any of the 4 species of nematodes was, by itself, capable of producing typical soreshin lesions. Only in several instances was there evidence of an increase in the number and severity of the hypocotylar lesions produced by the several species of fungi when associated with nematodes over those produced by each of the fungi alone.

*Fusarium moniliforme* tended to reduce the total germination of the seed, relative to that of the control, from 10 to 15 per cent, and to increase greatly the number of lesions on the hypocotyls. In no instance did it produce typical damping off, although the plants developing in the cultures containing this fungus were invariably very irregular in size—results comparable to those reported by Woodroof.<sup>2</sup> The addition of nematodes to cultures in

<sup>2</sup> Woodroof, N. C. A disease of cotton roots produced by *Fusarium moniliforme* Sheld. Phytopath. 17: 227-238. 1927.

which this fungus had been placed did not significantly increase the number and severity of diseased seedlings. In the 1935 series of experiments, however, in which the plants were grown for 2 months, then harvested and weighed, the combination of this fungus with each of the several species of nematodes reduced the average weight of the plants relative to those grown in cultures containing the fungus alone as follows: *C. elongatus*, 14 per cent; *A. parietinus*, 30 per cent; *A. avenae*, 38 per cent; and *A. bütschlii*, 30 per cent. The best plants in these cultures were as large as the best plants in the control cultures (no nemas nor fungi). The reduced average weight was largely due to the number of stunted plants, which were fewest in the cultures containing the fungus alone.

*Fusarium vasinfectum* tended slightly to decrease the germination of the seed and increase the number of hypocotylar lesions, but it did not show this unfavorable effect in all experiments. In the 1936 series of experiments the nematodes greatly increased the number of hypocotylar lesions, which were much more numerous at soil temperatures of 24° and 27° C. in the cultures containing both the nematodes and this fungus than in the cultures containing this fungus alone. At a soil temperature of 21° C., there was little difference between the two types of cultures. In the 1935 and 1936 series of experiments, 60 and 30 per cent, respectively, of the plants in the cultures containing this fungus were infected by it—some of the plants being killed in the seedling stage, others showing typical wilt after a growth period of 3 months. The amount of wilt was not influenced by the addition of nematodes to the cultures.

*Glomerella gossypii* caused typical damping off in all experiments. In the 1935 series only 28 per cent of the original seedlings (germination 80 per cent of that of the controls) were alive after 9 weeks, and most of the surviving plants were small and diseased. As in the wilt-fungus cultures, the severity of seedling injury was not appreciably greater when the nematodes were present in the cultures.

As reported earlier,<sup>3</sup> in the 1935 series of experiments, where *Aphelenchoides parietinus* was present, malformed terminal buds developed on 5 to 25 per cent of the seedlings when the soil temperature was sufficiently low to delay the emergence of the cotyledons from the soil to 5 or more days after planting.

One is probably not justified in concluding that the presence of these nematodes in the hypocotylar lesions has no effect on the plants. Such a conclusion would scarcely seem tenable in view of the fact that the nematodes sometimes migrate from the diseased area into adjacent cortical tissue. It also appears that the presence of the nematodes in the soil in large numbers may sometimes have a stunting effect on the plants. At least *Aphe-*

<sup>3</sup> See footnote 1.



*lenchoides parietinus* feeds to some extent on the surface of the roots. However, the experiments failed to indicate that the 4 species of nematodes investigated are factors of primary importance in the etiology of damping off, or soreshin, in cotton.

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## SEROLOGICAL REACTIONS FOR THE DETERMINATION OF BACTERIAL PLANT PATHOGENS

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There has been a growing tendency among certain plant pathologists in the last decade to turn to serological reactions for the determination and classification of bacterial plant pathogens. The word specificity is being employed, with its implication that here one has the key to all species. Serological reactions are being advocated and used for the plant pathogens despite the fact that no careful and extensive investigation has been made on a single well-known species to determine what may be expected to occur under the various tests. The agglutination and precipitin reactions have been used by the plant pathologist, although the bacteriologist usually considers the agglutinin absorption and the complement fixation reaction more delicate. The agglutination test is simple but has the disadvantage that many bacteria agglutinate under certain environmental conditions without the presence of antibodies. The precipitin test is employed mainly for the determination of proteins, and, since many overlapping reactions occur, even with the pure substances, this test might tend to bring together species. Furthermore, in certain cases (4) it has been shown that the proteins are the group antigens and that polysaccharides are serologically active in separating strains. Consequently, it would appear that these and other points should be considered well before one could use the tests with any assurance.

What constitutes specific characters in bacterial plant pathogens is possibly involved in the discussion as to whether or not these methods are applicable. Stapp (6), an advocate of serological methods, states: "When precipitation reactions are positive, identity of the species is unequivocally confirmed, even though there be definite cultural and physiological differences." To what extent he would allow physiological differences to exist in



a species, he does not state, but the statements is very broad. Why one should hold to serological properties and allow other physiological characters to vary is difficult to understand. In the light of recent work in bacteriology and serology it would be more logical to separate a species on the basis of stable physiological characters, and allow the serological reactions to vary. The bacteriologists generally do this and pay little attention to serological variations, unless they are a good key to some important properties otherwise difficult to determine, as in types I, II, and III, and group IV of the *Pneumococcus*.

In certain genera of bacteria considerable work has been done in the field of serology. What then is the opinion of some of these investigators in regard to species determination by serological methods? Topley and Wilson, v. 1, p. 221 (8), make the general remark without naming genera or species: "In certain groups of bacteria, the serological method is found to be the quickest and most satisfactory way of distinguishing between the different members and it is therefore extensively used for rapid identification. In other groups the agglutination method is not of much help." Stevens (7) worked with many isolates of *Rhizobium*, and states: "The results of these observations indicate the impossibility of identifying an organism by means of the agglutination tests." And Fred, Baldwin, and McCoy (1), in their monograph on this genus, say that "serological reactions have only a limited value in species identification, but they are of the utmost value in strain identification and classification." Hucker (3) in this extensive work on the *Coccaceae* states that "the wide variation among individual strains together with a lack of correlation between the serological and other biological characters, suggests that groups formed on a serological basis alone are not natural species." Hall (2), in discussing the classification of bacteria, remarks that "there is no reason for basing binomial subdivisions upon serologic agglutination distinction." He cites *Bacillus welchii* and states that there "are few species of bacteria now so well defined in their essential characters" as this organism, yet it is composed of many serological strains. And finally, Kennedy (5), in leaflet 8, Ed. 4, 1935, on serological reactions prepared for the Manual of Methods for Pure Culture Study of Bacteria, remarks that these "reactions often have a peculiar value not as substitutes for those to be gained from morphological, cultural, or biochemical means, but as supplemental to them."

In plant pathology the investigator is interested in the pathogenicity of a bacterium on a certain plant, its host range, and its morphological and physiological characteristics in culture. It is yet to be shown in the genera *Erwinia* and *Phytophthora* that serological reactions are definitely correlated with these biological characters. Also, if serological strain differences exist among the plant pathogenic species, as very likely they do, it first must be

shown to what important pathogenic characters they are linked before they may be of real use.

The writer does not wish to be placed in the position of discouraging work on serology of the plant pathogens, for there is always the possibility of important contributions arising from such investigations. However, he does feel that until careful and extensive work has been conducted within the field to establish facts, one should be slow in placing too much emphasis on serological reactions among the plant pathogens.

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# THE EVALUATION OF SOME CUPROUS OXIDES RECOMMENDED AS SEED-TREATMENT PRODUCTS FOR THE CONTROL OF DAMPING OFF<sup>1,2</sup>

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(Accepted for publication March 9, 1937)

Since attention has been directed by Horsfall<sup>3</sup> to cuprous oxide as a means of controlling damping off several manufacturers have offered cuprous oxide products for the purpose. The products offered vary greatly in appearance and at once raise the question of their relative value as seed treatments. Tentative specifications of cuprous oxide dusts have been listed by Horsfall *et al.*,<sup>4</sup> but preliminary trials at Urbana, Illinois, under greenhouse conditions indicated that certain of these were in need of revision. Materials which have become available since Horsfall's specifications were published have made additional comparisons of cuprous oxides desirable. Accordingly, this study was made to compare and evaluate the cuprous oxides manufactured and sold as treatment materials for the control of the pre-emergence phase of damping off.

The division of the disease into pre- and postemergence phases has served to emphasize the fact that poor stands do not, as a rule, indicate poor seed germination, but instead, that the young tender seedlings have been killed before emerging from the soil (see Fig. 1). Any grower of seedlings is familiar with the postemergence phase, which is much more evident as a disease because the seedlings are killed after emergence (Fig. 2).

## EXPERIMENTAL TESTS NO. 1 AND 2

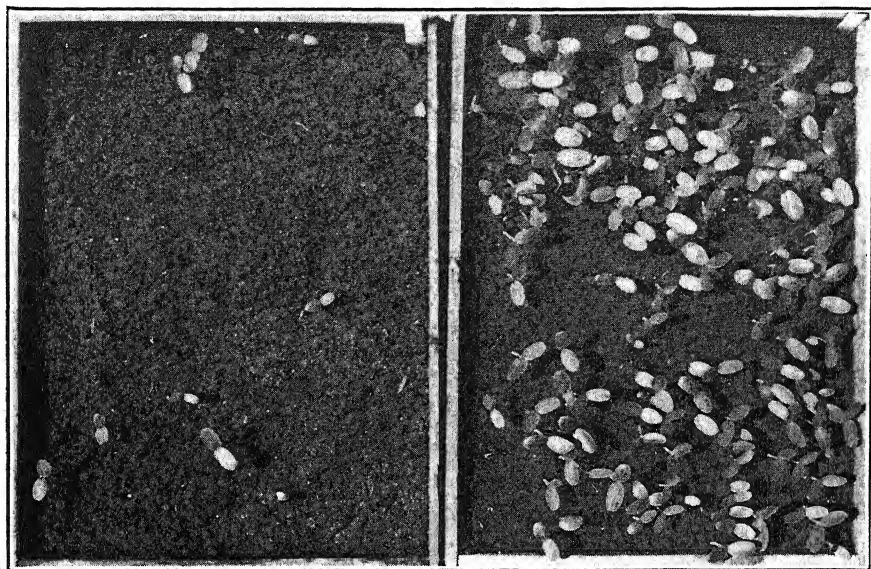
The first comparisons of the available cuprous oxides were made in the springs of 1934 and 1935 at Urbana, Illinois, in greenhouses of the Horticulture Department. The soil used was known to be heavily infested with damping-off species of *Pythium* and *Rhizoctonia*. It was a black silt loam, high in humus, which had been used many years for vegetable culture. During the course of this study the soil temperature was maintained between 72° and 76° F., while the temperature of the greenhouse ranged between 75° F.  $\pm$  7°. The soil moisture was kept at medium to high by watering daily.

<sup>1</sup> Research project administered by Crop Protection Institute.

<sup>2</sup> The cost of publication of this paper was defrayed by the Crop Protection Institute.

<sup>3</sup> Horsfall, J. G. Red oxide of copper as a dust fungicide for combating damping-off by seed treatment. New York (Geneva), Agr. Exp. Sta. Bul. 615. 1932.

<sup>4</sup> ———, Newhall, A. G., and Guterman, C. E. F. Dusting miscellaneous seeds with red copper oxide to combat damping-off. New York (Geneva) Agr. Exp. Sta. Bul. 643. 1934.



(Photo courtesy Ill. Agr. Exp. Sta.)

FIG. 1. Premergergence damping-off of cucumbers and its control by cuprous oxide seed treatment. The same number of seeds were planted in each of the above flats of unsterilized garden soil. Seeds in the flat to the left were nontreated and all but 9 seedlings were killed before they emerged from the soil. Seeds in the flat to the right were treated with cuprous oxide and nearly all that were planted have produced healthy vigorous seedlings. Proper seed treatments are very effective in controlling this phase of damping off.

The materials tested were:

“Cuprocide”—a cuprous oxide submitted by Röhm and Haas Chemical Co., bright or carmine red in color. Smooth, even powder.

Cuprous oxide—submitted by The Mallinckrodt Chemical Co., bright or carmine red. A powder, slightly lumpy or oily.

Cuprous oxide—submitted by The Merck Chemical Co., bright or carmine red. A powder, slightly lumpy or oily.

Cuprous oxide—submitted by The Ansbacher-Siegle Co., purplish with about 25 per cent active ingredient. Smooth, even powder.

The materials were added in excess, and whatever did not adhere to the seed was screened off. This practice was followed in order to give each material every possible chance as a seed treatment and also to accentuate injury.

In each treatment 250 seeds were used for each planting. The results are listed in table 1 as the percentage total emergence (first figures) and percentage postemergence damping off (figures after hyphen). The pre-emergence damping off may be figured by subtracting total emergence from the germination of the seed, which also is listed. The crops used were beet, Swiss chard, cucumber, tomato, eggplant and spinach.

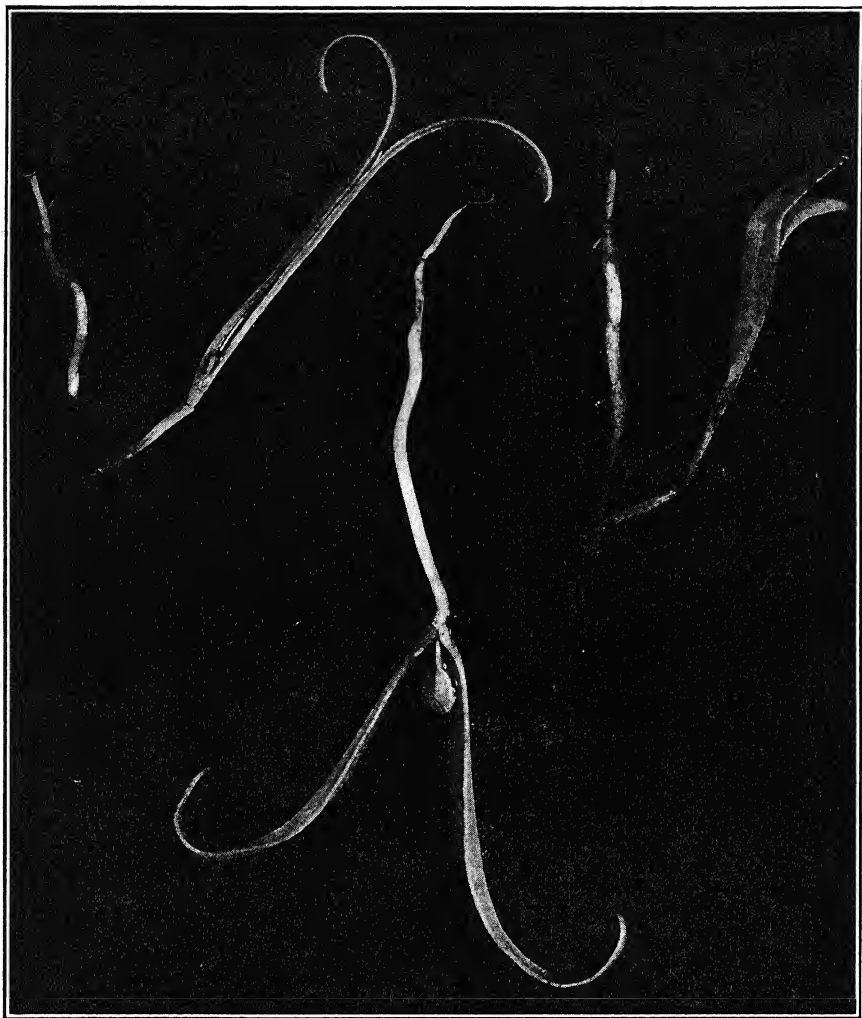


FIG. 2. Postemergence damping off of spinach seedlings. Typical effects of post-emergence damping off are shown in the two outside seedlings; the center plant is healthy. This phase of the disease, though better known to most growers, is less common than the preemergence phase. Seed treatments are not effective methods of controlling this phase of the disease.

The differences between the products submitted by Röhm and Haas, Mallinckrodt, and Merck Chemical companies are not considered significant, in spite of the fact that the Röhm and Haas "Cuprocide" imparted a more desirable type of coverage to the seed. All three of these products are essentially pure cuprous oxide. The Ansbacher-Siegle Company's product, which

TABLE 1.—Comparative value of cuprous oxides recommended for damping-off control greenhouse studies<sup>a</sup>

Crop	Year	Seed germination	Non-treated Checks	Röhm & Haas Chem. Co.	Mallinek-rodt Chem. Co.	Merck Chem. Co.	Ansbacher-Siegle Chem. Co.	Comments
Cucumber	1934	100	57-11 <sup>b</sup>	100-0	94-0	96-0	79-2	Röhm & Haas and Ansbacher-Siegle products stuck uniformly and well. The other two gave uneven, slightly bumpy coverage.
	1935	99	45-8	98-0	96-1	95-0	53-1	
Beet	1934	99	47-24	148-11	141-9	143-6	97-12	All treatments stuck well—germination on basis of seed balls only—multiple sprouts not counted.
	1934	92	70-6	134-3	137-2	124-1	78-1	
Tomato	1934	91	36-2	88-3	83-1	86-3	41-0	All materials stuck uniformly and well.
	1935	88	44-6	81-2	80-1	79-4	25-2	
Eggplant	1934	98	43-10	91-2	93-4	89-3	44-4	Röhm & Haas and Ansbacher-Siegle products stuck uniformly and well. Mallinek-rodt & Merck products gave heavy bumpy coverage.
	1935	98	47-10	90-2	95-4	89-4	46-1	
Spinach	1934	86	11-10	83-16	79-11	78-12	20-3	Coverage as for cucumber.
	1935	79	41-10	77-5	76-4	71-6	43-4	

<sup>a</sup> Soil pH 6.5—moisture medium to high; soil temperature 72°-76° F.; humidity variable, usually between 50 and 75 per cent.<sup>b</sup> Figures to the left of hyphen equal percentage total seedling emergence; figures to the right equal percentage postemergence damping off.

TABLE 2.—Comparative value of cuprous oxides recommended for damping-off control greenhouse studies<sup>a</sup>

Treatments	Lettuce	Carrot	Beet	Swiss chard	Onion	Cucumber	Muskmelon	Watermelon	Tomato	Pepper	Eggplant	Spinach	Comments
Nontreated checks	60-1 <sup>b</sup>	33-2	40-19	55-30	71-8	39-17	4-0	22-0	50-2	48-0	47-2	24-12	Disease caused principally by <i>Pythium</i> spp. although <i>Rhizoctonia</i> was involved in many instances.
Röhm and Haas Chemical Co.	78-1	48-6	87-12	129-15	72-10	90-11	69-5	91-1	86-1	50-0	62-2	61-16	Excellent, smooth, even coverage of all seed, except carrots and onions, which were poorly covered.
Mallinckrodt Chemical Co.	87-2	47-5	83-20	117-13	65-9	91-3	45-4	86-1	85-0	59-0	67-1	58-7	Coverage on most smooth-coated seed uneven and lumpy; on others, coverage heavy and lumpy.
Ansbacher-Siegle Chemical Co.	81-4	45-2	59-11	106-36	70-8	88-6	17-1	48-1	78-1	54-0	59-3	48-11	Excellent coverage of all seed.
Metals Refining Company	87-1	49-3	97-16	139-18	68-5	91-8	70-4	95-6	80-1	57-0	56-3	61-15	Excellent, smooth, even coverage of all seed, except carrots and onions, which were poorly covered.
Seed Germination	87	60	74 <sup>c</sup>	93 <sup>c</sup>	80	97	89	85	96	62	77	69	Tests run in laboratory.

<sup>a</sup> Soil pH 6.5—moisture medium to high; soil temperature 72°-76° F.; humidity variable, usually between 50 and 75 per cent.<sup>b</sup> Figures to the left of hyphen equal per cent total seedling emergence; figures to the right equal post-emergence damping-off.<sup>c</sup> Germination of seed balls only.

is only partially cuprous oxide, was decidedly inferior to the other three in all cases; in fact, crops treated with it were little better than the nontreated checks.

#### EXPERIMENTAL TESTS NO. 3 AND 4

These tests were conducted under conditions almost identical to those of tests 1 and 2 already discussed. The principal difference was about a 2° F. raise in both air and soil temperatures.

Because of the lack of material and the similarity in appearance and performance of Mallinckrodt's and Merck's cuprous oxides, the product of the Merck Chemical Co. is not included in these tests. In addition to the cuprous oxides of Röhm and Haas, Mallinckrodt, and Ansbacher-Siegle Chemical Companies, "Metrox," a cameo brown cuprous oxide, submitted by the Metals Refining Company, was tested. Like "Cuprocide" it is a smooth, even-texture powder.

As before, the materials were added in excess and whatever did not adhere to the seed was screened off.

The crops included in these tests were lettuce, carrot, beet, Swiss chard, onion, cucumber, muskmelon, watermelon, tomato, pepper, eggplant and spinach. Two hundred and fifty seeds of each crop were treated with each material tested.

The results of these tests are listed in table 2. They represent the average in per cent of 4 different plantings.

In these tests the cuprous oxides of Röhm and Haas, Mallinckrodt, and the Metals Refining Companies gave equally satisfactory control of damping off, since the differences between them were not considered significant. Again, the Ansbacher-Siegle product was decidedly inferior to the others tested. All the materials, except Mallinckrodt's, gave an equally smooth, uniform coverage to all seed except carrot and onion. Mallinckrodt's cuprous oxide was inclined to give a heavy, lumpy, uneven coverage.

#### FIELD TESTS

In the tests thus far completed, the cuprous oxides of Röhm and Haas, Mallinckrodt, Merck and Metals Refining Companies all gave about equally satisfactory control of damping off. The product of the Ansbacher-Siegle Co. was of little value in controlling the disease. Cuprocide of Röhm and Haas and Metrox of the Metals Refining Co. imparted the most satisfactory coverage to the seed. Because of these facts the only cuprous oxides included in the following field tests are Cuprocide and Metrox.

One test was conducted with the aid and on the farm of P. Fournie & Son, general vegetable growers northwest of Collinsville, Illinois. The soil was of the loess type with a pH of 5.8. The plantings were drilled in and



the results adjusted to the various seeding rates induced by the different chemicals used. Both Cuproicide and Metrox were used at the rate of four-tenths of an ounce to one pound of carrot and spinach seed. On beets they were used six-tenths of an ounce to one pound of seed. These are the concentrations recommended by Röhm and Haas Chemical Co. in connection with the use of their product, Cuproicide.

The results of the test are listed in table 3 and indicate that both Cuproicide and Metrox are excellent materials for the control of damping off.

TABLE 3.—*A field comparison of Cuproicide and Metrox on farm of P. Fournie & Son, Collinsville, Ill.*

Crop	Relative stands			Comments
	Checks	Cuproicide	Metrox	
Carrots <sup>b</sup> .....	59	132	130	Records from 25 feet of row. Average number of plants from 10 different counts.
Beets <sup>c</sup> .....	25	417	436	Records from 99 feet of row. Average number of plants from 2 different counts.
Spinach <sup>c</sup> .....	132	557	608	Records from 99 feet of row. Average number of plants from 2 different counts.

<sup>a</sup> Soil pH 5.8—Results adjusted to different seeding rates.

<sup>b</sup> Soil remained quite dry for 1½ weeks after carrots were planted and then heavy rains fell followed by warm temperatures.

<sup>c</sup> 16 hours after the beets and spinach were planted heavy rains fell followed by warm temperatures.

The other test was run under the overhead irrigation system of the Horticulture Department, University of Illinois, Urbana, Illinois. The treatments were Cuproicide and Metrox and were applied according to the recommendations of Röhm and Haas in connection with the use of their product, Cuproicide. The plantings were repeated from two to four times, except for one large planting of spinach which was not under irrigation. In all cases the actual number of seed planted was known. This number is given in the table of results under each crop. The results are listed in per cent and represent the average of all plantings in this test. The number of planting of any particular crop was always the same for each treatment. The pH of the soil was 6.5. Except for the large spinach planting the plots were watered daily.

The results are listed in table 4 and again indicate that both Cuproicide and Metrox are excellent treatments for the control of damping off. The

TABLE 4.—*A field comparison of Cuprocide and Metrox, University Farm, Urbana, Ill., 1936<sup>a</sup>*

Crops and number of seed planted	Final stand in per cent				Comments
	Nontreated checks	Cuprocide	Metrox	Germination of seed	
Lettuce 500	52	57	60	75	Excellent coverage.
Carrot 500	44	55	57	61	Poor coverage by both materials.
Beet 1000	33	71	63	62 <sup>b</sup>	Excellent coverage.
Swiss chard 1000	62	145	126	91 <sup>b</sup>	Excellent coverage.
Pea 500	40	87	83	99	Excellent coverage.
Onion 500	66	69	70	92	Poor coverage by both materials.
Cucumber 1000	39	87	84	98	Light spotted coverage.
Muskmelon 1000	13	56	70	85	Light spotted coverage.
Watermelon 1000	17	90	89	96	Light spotted coverage.
Squash 1000	47	62	63	97	Light spotted coverage.
Tomato 500	66	69	73	88	Excellent coverage.
Eggplant 500	32	53	44	75	Light spotted coverage.
Spinach 500	12	29	30	59	Excellent coverage.
Spinach not under irrigation 6077	16	64	63	59	Excellent coverage.

<sup>a</sup> Experiment under irrigation—watered daily—soil pH 6.5.

<sup>b</sup> Figures indicate per cent of seed balls that germinated—individual seeds of ball not counted. In all cases germination of seed was determined in laboratory tests.

materials are particularly valuable on those crops which are especially susceptible to preemergence damping off.

#### Preservation of Pea Seed by Treatment

A finding of considerable interest, observed in these studies, was the indirect effect of seed treatment on subsequent growth and vigor of pea seedlings. Because of the very apparent difference between the growth of the seedlings from treated and nontreated pea seeds, a careful examination of the plantings was made. It was found that, even though the plants from nontreated seeds appeared healthy in most cases, the cotyledons that remain below the ground and furnish food and minerals to the growing seedling were nearly always completely disintegrated. In the case of treated seed they were usually healthy and still attached to the plant, although greatly

shriveled, indicating that the food and mineral supplies were about used up. There was a definite correlation between seedling size and vigor and the general condition of the cotyledons. As a check on this finding, plantings of nontreated and Metrox-treated peas were made in the same soil and grown in pots in the greenhouse. The results of this test were identical to our field observations and are illustrated in figures 3 and 4. The peas were of the Surprise variety.

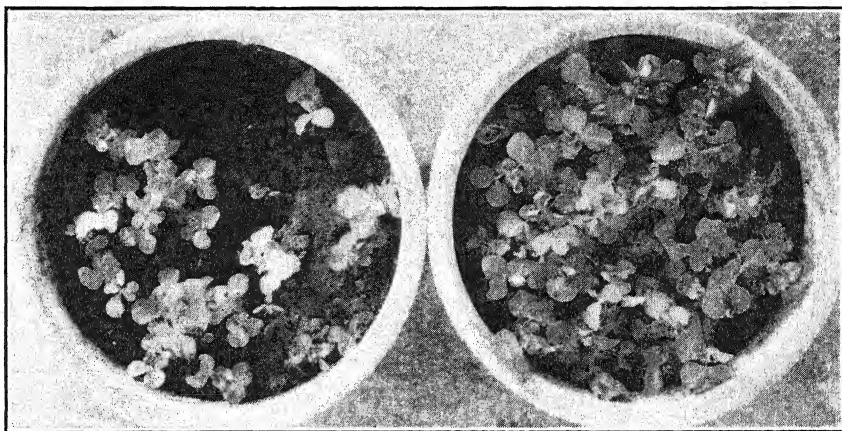


FIG. 3. Control of damping off of peas by cuprous oxide. 100 seeds of the Surprise variety were planted in each pot of garden soil. Those in the pot to the left were nontreated and 23 came up; the seed in the pot to the right were treated with Metrox (cuprous oxide) and 94 came up. Both were planted at the same time. Note the apparent difference in vigor.

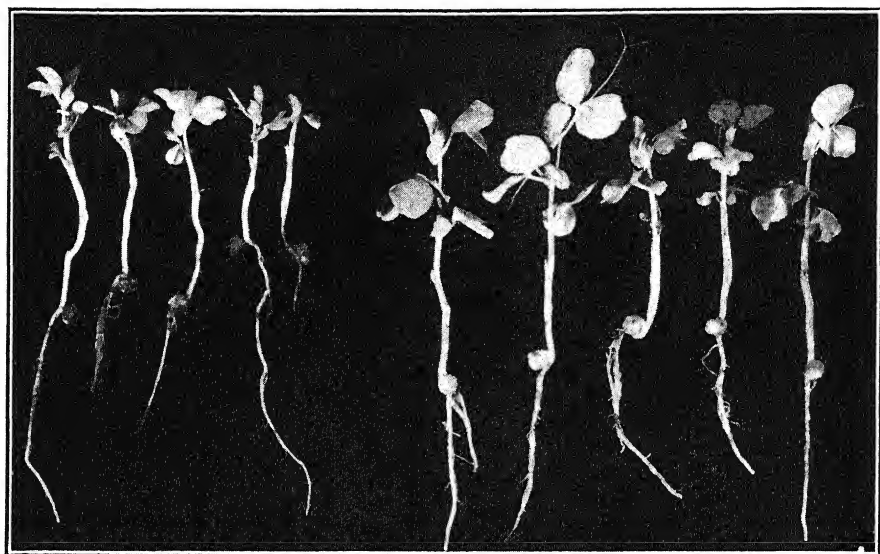
Thus the benefits of seed treatment may be enumerated to include increased vigor of pea seedlings. By preventing the cotyledons from rotting, it is possible that subsequent stem rots, so common on peas in Illinois, may be materially reduced.

#### Seed Coverage

Another study of general interest in determining the specifications of a satisfactory cuprous oxide or any other seed treatment concerns the relation of seed coverage to damping-off control.

Of all the materials tested at Urbana during the last four years, the only ones that generally gave good coverage on the crops for which they were recommended were Semesan (an organic mercury) and certain special makes of zinc and cuprous oxide. Materials that do not give consistently good coverage cannot be relied upon as seed treatments.

The following experiment serves to illustrate this point. It also seems to illustrate that proper seed coverage, not too much nor too little, is more



(Figures 3 and 4 by courtesy Ill. Agr. Exp. Sta.)

FIG. 4. Effect of seed treatment upon the cotyledons and growth and vigor of peas. The seedlings on the left were taken from the nontreated pot pictured in figure 3. The cotyledons are badly rotted and in some cases the stem is invaded at the point of attachment. The seedlings to the right were taken from the pot to the right in figure 3. They were treated with cuprous oxide and, aside from being badly shriveled, are in perfect condition. Note the difference in the size of the seedlings.

important than is the copper compound used. Watermelon seeds were selected for the experiment because few materials stick well to any of the cucurbits and the seedlings of the entire group are especially susceptible to damping off.

The materials used were Cuprocide, Metrox, Semesan, copper carbonate and cuprous oxide. The first three stick fairly well to cucurbits and were applied to dry seed only; but to get good coverage with the last two, it was found necessary to moisten the seed. The experiment was conducted at two different times under greenhouse conditions. The temperature of the house varied between 75° and 85° F. The soil moisture was medium to high and the pH was 6.5. The results are given in table 5 as the actual number of seedlings from 250 seeds planted in each of two plantings.

The value of proper seed coverage is well brought out in the table. In the second planting the seeds were a little too moist and so much material adhered that considerable injury resulted from both copper carbonate and cupric oxide. This fact probably accounts for the reduced emergence in the second planting as compared to the first. While this experiment should not be regarded as conclusive, it does indicate that even cupric oxide has con-

TABLE 5.—*Relation of seed coverage by various copper compounds to damping-off control<sup>a</sup>*

Treatment	Total emergence	Damping-off Post-emerg.	Comments
	7	— 0	
Nontreated checks .....	23	— 0	Planted in black silt loam garden soil.
	1	— 0	Planted in typical sandy water-melon soil noninoculated.
	51	— 0	
Metrox—	222	— 9	Good coverage. Seed in silt loam soil.
(cuprous oxide) .....	239	— 2	
Semesan	209	— 0	Very good coverage. Seed in silt loam soil.
(organic mercury) .....	226	— 2	
Cuprocide—	213	— 2	Good coverage. Seed in silt loam soil.
(cuprous oxide) .....	214	— 2	
Copper carbonate .....	73	— 8	Poor spotty coverage. Seed in silt loam soil.
Seed dry .....	131	— 4	
	222	— 5	Heavy coverage. Injury in second planting. Silt loam soil.
Seed moist .....	135	— 4	
Cupric oxide .....	13	— 0	Very poor coverage. Silt loam soil.
Seed dry .....	5	— 0	
	205	— 5	Heavy coverage. Injury in second planting. Silt loam soil.
Seed moist .....	91	— 5	

<sup>a</sup> Results listed as actual number of plants involved. Two hundred fifty watermelon seeds were planted in each instance, having a germination of 96 per cent (240 seeds).

siderable fungicidal value as a seed treatment for damping-off control when proper coverage of the seed is obtained. Additional evidence in support of this deduction was secured by including cupric oxide on a few crops in the Urbana field test discussed on page 581. Beets, Swiss chard and spinach were treated with Cuprocide, Metrox, Semesan, cupric oxide, Vasco 4 and Leafox. All materials stuck well to the seed of the three crops except cupric oxide, which stuck very poorly to spinach. None of the seeds were moistened as in the above experiment with watermelon seed. The results are listed in table 6 in terms of percentage.

Here again, it is clearly evident that cupric oxide, although inferior to cuprous oxide, has considerable fungicidal value when it adheres properly to the seed. Thus, the possible conversion of cuprous oxide to cupric is of concern more from the standpoint of subsequent sticking qualities than from the fact that a new form of copper is involved. Through correspondence with G. H. Brown it has been learned that some limited studies by Mallinckrodt chemists indicate that coverage with zinc oxide is very closely related to seed and air moisture. The higher the moisture the better the adhesion. Whether or not this finding is applicable to all treatments has not been determined, but the very fact that the type of coverage from all treatments varies from time to time indicates a possible connection.

TABLE 6.—*Relation of seed coverage to damping-off controls*<sup>a</sup>

Crop	Checks	Cupro- cide	Metrox	Cupric oxide	Semesan	Vasco 4	Leafox	Seed germi- nation
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Beet <sup>b</sup> .....	23	66	69	53	39	42	52	62
Swiss chard <sup>b</sup> ...	48	146	113	100	123	68	84	91
Spinach <sup>b</sup> ...	16	35	36	18	27	33	33	59

<sup>a</sup> Results figured from a 1000-seed planting of Swiss chard and beet and two 500-seed plantings of spinach. Not comparable to data in table 4, which represent an average of several plantings.

<sup>b</sup> Germination of seed balls, not individual seeds of ball.

#### DISCUSSION

The relative merits as seed treatments for damping-off control of five different makes of cuprous oxide have been studied, with the object of learning the necessary specifications of a satisfactory treatment. Good commercial control of damping off of several different vegetable crops was secured by seed treatment with cuprous oxides of Röhm and Haas, Mallinckrodt, Merck, and Metals Refining Companies. The dusting and adhesive properties of Cuprocide (Röhm and Haas) and Metrox (Metals Refining Co.) are such as to make these products more desirable than the other two as seed treatments. The product of the Ansbacher-Siegle Co. was ineffective for seed treatment of most crops, although its physical properties were very satisfactory. The low percentage (25 per cent) of active ingredient seems to account for its failure to control preemergence damping off. The other four materials tested were very nearly pure cuprous oxide.

The color of cuprous oxide is an unreliable index of treatment value for damping-off control. A considerable number of copper compounds are effective as seed treatments, provided they give *adequate coverage* to the seed in question and contain a *large amount of copper*. Limited data are presented to illustrate this point.

Attention is directed to the fact that proper seed treatment of peas, in addition to damping-off control, practically prevents the rotting of the cotyledons, which is reflected as increased vine growth and vigor.

#### Specifications of Cuprous Oxides to be Used as Seed-treatment Materials for the Control of Preemergence Damping Off

1. The product in question should contain no less than ninety-five per cent cuprous oxide.
2. The treatment should impart a smooth, uniform coverage to the treated seed when used at recommended amounts. If the material is fluffy, flows evenly, and fumes or smokes when shaken, it may be regarded as dustable. As a test of adherence, other workers have suggested placing small amounts

on a clean white piece of paper and then slowly turning it to a vertical position; enough dust should remain to color the paper well.

3. The material should be fine enough to pass a 325 mesh screen. Additional fineness does not improve its fungicidal values. (Data not presented.)

#### PRECAUTIONS

No copper treatments should ever be used in Illinois on the seed of crucifers; they are almost certain to cause injury.

Cuprous oxides that contain more than 5 per cent inert ingredients should not be used unless recommended by an unbiased authority.

Cuprous oxide should not be used in soil that is more acid than pH 5, since injury often results under such conditions. In fact, if such a condition exists, the soil should be sweetened by liming.

All chemicals should be used EXACTLY as recommended by the manufacturer. Because of local conditions a list of crops to be treated should be secured from the State or local authorities, if best results are desired.

Seed treatments will not control postemergence damping off, unless temperature and humidity are properly regulated.

Pea seed, inoculated with nodule-producing bacteria, should not be treated with any chemical, since those chemicals that are effective in damping-off control will kill the nodule bacteria on the seed. Seed treatment does not effect nodulation if the bacteria are present in or are added directly to the soil.

Seed treatment materials are POISON and should be kept out of the reach of children and animals.

If treatments are used for the control of damping off of crops grown under glass, they must be supplemented by proper cultural practices. Watering should be held at a minimum after the seeds are planted. Good aeration and low humidity should be provided. The temperature should be maintained at a level most favorable to the crop being produced.

Under Illinois conditions either *Metrox* or *Cuprocide* is recommended for damping-off control of the following crops and should be applied according to the manufacturer's recommendations. Crops are listed in the general order of benefits received by cuprous oxide treatment: watermelon, cucumber, spinach, Swiss chard, beet, muskmelon, tomato, pea,<sup>5</sup> eggplant, pepper, squash, lettuce, and endive.

Most other vegetables grown in this State either do not respond to treatment or are benefited more by other chemicals. For details, write to the Illinois Agricultural Experiment Station, in care of the senior author.

<sup>5</sup> Graphite is recommended to prevent clogging of drills when sowing cuprous oxide dusted pea seed. (New York (Geneva) Agr. Exp. Sta. Bul. 660, 1936.) It may also be used to advantage in connection with cuprous oxide treated spinach seed and should be used at 1% by weight with all crucifer seeds that have been treated with some form of zinc oxide. (Correspondence with Dr. H. T. Cook, 12/9/36.)

## PHYTOPATHOLOGICAL NOTE

“*Spore mats*” of *Phymatotrichum omnivorum*.—While studying “spore mats” of cotton root rot in the field at Sacaton, Arizona, it was found that better mats for study could be obtained if they were protected from the sun, wind, and insects. The “spore mats” begin as white, cheesy masses of mycelia. These were covered carefully as they began to develop, and within a week the supporting cells had broken down and the mat consisted mostly of a powdery mass of spores. Upon close examination the whole mat at this stage is seen to be covered by a loose felt or membrane of hyphae. The *Phymatotrichum* spore mat would thus become not a Hyphomycete fruit mass but a closed fruit body, suggesting in general appearance a *Gasteromycete*, or some obscure accessory structure of an *Ascomycete*.—JOHN T. PRESLEY and CHARLES THOM.



# STUDIES ON THE TRANSMISSION OF PEA VIRUS 2 BY APHIDS<sup>1</sup>

H. T. OSBORN

(Received for publication January 27, 1937)

In the course of experiments on the incubation period of pea-mosaic virus<sup>2</sup> in the pea aphid, *Macrosiphum pisi* Kaltenbach, it was found that there are several viruses capable of causing disease in peas (7). Subsequently (8) one of these diseases, caused by a virus designated as pea virus 2, was found to be transmitted without the necessity of an incubation period in the pea aphid. Thus the method of transmission by an insect vector served to distinguish two virus diseases. Since little attention has been given to the manner in which aphids transmit plant-virus diseases, it seemed of interest to determine the method of transmission of pea virus 2 not only by the pea aphid but also by any other aphid vectors that might be found. The potato aphid, *Macrosiphum gei* Koch, and the bean aphid, *Aphis rumicis* L., as well as the pea aphid, were found to transmit pea virus 2 and, being easy to maintain in culture, were used in studies on transmission. Mechanical inoculations were employed in experiments made to determine certain properties of the virus.

The object of this paper is to present results of experiments on the method of transmission of pea virus 2 by aphids and results of studies on certain properties of the virus.

## REVIEW OF LITERATURE

An incubation period was shown by Osborn (7, 8) for pea virus 1 in the pea and potato aphids. Elze (4) and Smith (10) found that the virus of potato leaf roll undergoes an incubation period in the green peach aphid *Myzus persicae* Sulz. These are the only cases in which incubation periods have been demonstrated for plant viruses in aphid vectors.

On the other hand, it is known that certain plant-virus diseases do not require incubation periods in the aphids that serve as vectors. This has been shown by Doolittle and Walker (2) for transmission of cucumber mosaic by the melon aphid, *Aphis gossypii* Glover, and by Hoggan (6) for transmission of cucumber-mosaic virus to tobacco by the green peach aphid. Drake *et al.* (3) found 50 different species of aphids capable of carrying yellow dwarf of onion. No incubation period was demonstrated for any of these insects, and they lost the virus in a short period after removal from diseased plants. Zaumeyer and Kearns (12) recently secured transmission of bean mosaic

<sup>1</sup> Published at the expense of The Rockefeller Institute for Medical Research, Princeton, N. J., out of the order determined by the date of receipt of the manuscript. This practice in no wise delays the publication of manuscripts printed at the expense of The American Phytopathological Society or other agency.

<sup>2</sup> This virus is now referred to as pea virus 1 (9, 11).

with 11 species of aphids. Since these aphids lived for only a short time on bean, Zaumeyer and Kearns believed that they acquired the virus when they first fed on the diseased plants and that they were able to transmit it immediately to fresh plants, though no definite experiments were conducted to determine the presence or absence of an incubation period.

Doolittle and Walker (2) expressed the opinion that the virus of cucumber mosaic was carried into the plant tissues on the proboscis of the insect and that the minute amount of virus thus carried was exhausted during the first feeding period. Hamilton (5) fed *Myzus persicae* on media, one constituent of which was a radioactive indicator containing polonium, and observed that the insects picked up the indicator and transmitted it to a leaf. She observed further that a constant proportion of the amount imbibed was transferred to the host. From this observation she deduced that the polonium is transmitted through the bodies of the aphids and not on the outside of the stylets. She also presented evidence from which she concluded that virus probably is transmitted in the same way as polonium. However, her attempts to inoculate virus III of *Hyoscyamus* and Y-virus of potato, by feeding aphids on media containing these viruses, were not successful.

#### MATERIALS AND METHODS

The pea-mosaic disease used in these experiments was obtained from a naturally infected *Vicia faba* L. plant growing in a field near Princeton, New Jersey. The plant was stunted and distorted and proved to be affected by two distinct diseases. The virus obtained by mechanical transmission from this plant to healthy *V. faba* plants was found to be distinct from pea virus 1 and was designated as pea virus 2. It is transmitted readily by the pea aphid when no time is allowed for an incubation period, but is lost by the aphid soon after feeding on healthy plants. The virus transmitted from the naturally infected *V. faba* plant after a suitable incubation period in the aphid was pea virus 1. In order that pea virus 2 might be available at all times, it was maintained continuously in a greenhouse by successive transfers to fresh *V. faba* plants by the rubbing method of inoculation either with or without the use of carborundum powder and by insect inoculation.

Colonies of the pea and bean aphids were reared on healthy *Vicia faba* plants in screened cages in a greenhouse. The potato aphid was reared in like manner on plants of tomato, *Lycopersicon esculentum* Mill., or potato, *Solanum tuberosum* L. Several methods of handling aphids were used in making inoculations. For transfer of the disease when no experiment was involved, fresh plants were sometimes placed in a cage beside diseased plants on which aphids were colonized, thus permitting a natural migration from diseased to healthy plants. In other cases, stems from diseased plants on which aphids were feeding were cut off and placed on small healthy plants. When used in experiments, the aphids were transferred from plant to plant

with a camel-hair brush. They were first brushed onto a piece of paper, and then from the paper onto a fresh plant. Plants exposed to aphids for very short periods of time were kept under observation in a laboratory room. Plants exposed for long periods were isolated in lamp chimneys or in screened cages kept in a greenhouse. At the end of an exposure period the aphids were brushed off and the plants fumigated with Nicofume. The exposed plants were then taken to another greenhouse where they were grown beside noninoculated control plants. *V. faba* plants were used in the experiments, since they were found to be highly susceptible to the disease and in previous experiments had proved to be very satisfactory for use when rapid transfer of large numbers of aphids was desired. The plants for experimental use were grown in 2½-inch pots.

Resistance to heating was determined by placing 2 cc. of undiluted extract from diseased *Vicia faba* plants in thin-wall test tubes, heating in a water bath at the desired temperature for 10 minutes, and then testing for infectivity by mechanical inoculation to *V. faba* plants. For studying resistance to aging *in vitro*, 2 cc. of expressed juice from diseased plants was held in a test tube for the required period in a laboratory room and then tested for infectivity by mechanical inoculation to *V. faba* plants.

#### HOST RANGE AND DESCRIPTION OF THE DISEASE

Pea virus 2 was transmitted by mechanical inoculation from *Vicia faba* to the Telephone and Alderman<sup>3</sup> varieties of garden pea, *Pisum sativum* L., field pea, *P. sativum* var. *arvense*, sweet pea, *Lathyrus odoratus* L., crimson clover, *Trifolium incarnatum* L., red clover, *T. pratense* L., white sweet clover, *Melilotus alba* Desr., and the Green Stringless Refugee, Corbett Refugee, and Robust<sup>4</sup> varieties of garden bean, *Phaseolus vulgaris* L. By the same method it was transmitted back from each of these hosts to *V. faba*. The virus was transmitted from *V. faba* to pea, field pea, and Green Stringless Refugee bean by means of the pea aphid. It also was transmitted from red clover to *V. faba* by the same method.

Inoculations made to the garden pea variety Wisconsin Resistant Perfection,<sup>3</sup> both by means of pea aphids and by mechanical methods, failed to produce the disease. The garden bean variety Great Northern Idaho No. 1<sup>4</sup> failed to become infected in several trials by mechanical methods.

Attempts to infect white Dutch clover, *Trifolium repens* L., Turkish tobacco, *Nicotiana tabacum* L., *N. glutinosa* L., tomato, and mung bean,

<sup>3</sup> The Alderman and Wisconsin Resistant Perfection pea were tested for susceptibility following the work of Stubbs (11). Seed for the tests was secured through the courtesy of Dr. Merl W. Stubbs.

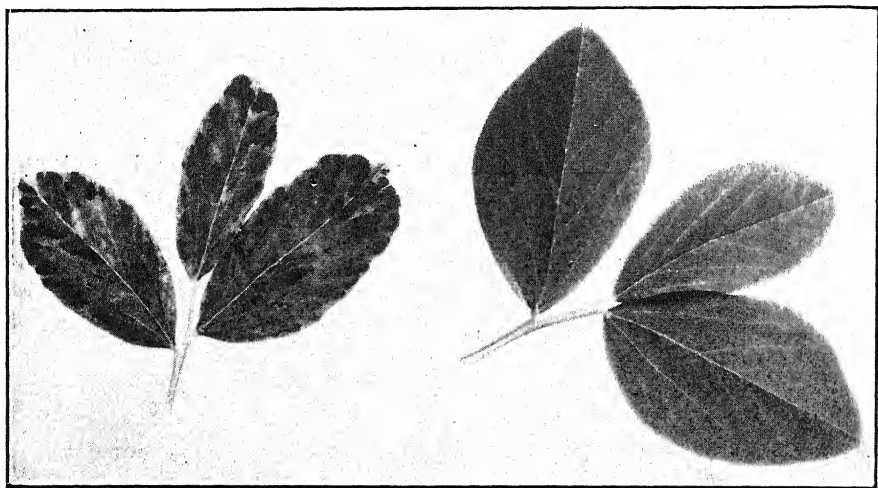
<sup>4</sup> The Green Stringless Refugee, Corbett Refugee, Robust, and Great Northern Idaho No. 1 beans are differential varieties suggested by Zaumeyer and Wade (13) for legume viruses. Tests of these varieties were made with seed secured from Dr. W. J. Zaumeyer.

*Phaseolus aureus* Roxb., by mechanical inoculations were not successful. Tomato plants were inoculated also by means of the potato aphid, but no evidence of mosaic disease was obtained.

The host range serves as an additional means of distinguishing between pea virus 1 and pea virus 2. Red clover, white sweet clover, and bean varieties, susceptible to pea virus 2, have failed to become infected with pea virus 1.

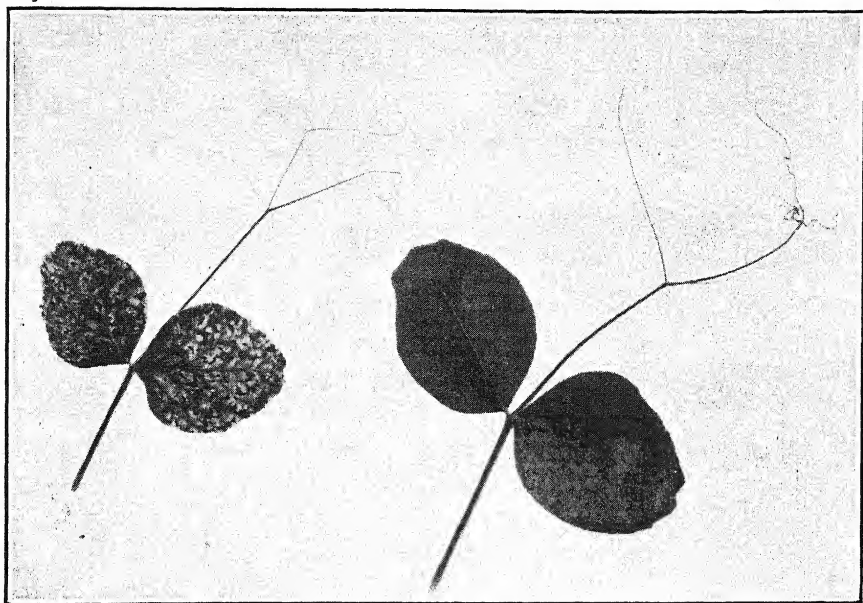
Pea virus 2 also may be distinguished from pea virus 1 by the symptoms produced on diseased plants. These have been described for pea virus 1 as a mosaic pattern consisting of a spotting of the leaves. Plants diseased with pea virus 2, on the other hand, are characterized by mottling or splotching of the leaves. No enations, such as are produced by pea virus 1 on the under sides of diseased pea and crimson clover leaves, have been observed on any plants infected solely with pea virus 2.

In *Vicia faba* the first symptoms caused by pea virus 2 usually appear within 6 to 10 days after inoculation as a spotting of the leaves or as a clearing of the veins. Diseased plants in later stages are characterized by a conspicuous mottling consisting of a grey-green background on which are superimposed sharply defined and strongly contrasted dark green splotches (Fig. 1). When this virus is transmitted to garden pea or field pea, a brilliant marking of the leaves is produced. An advanced stage of the disease on Alderman pea is shown in figure 2. Infected crimson clover plants are characterized by a brilliant mottling of the leaves, such as is shown in figure 3. Crimson clover plants are severely stunted. Growth of red clover,



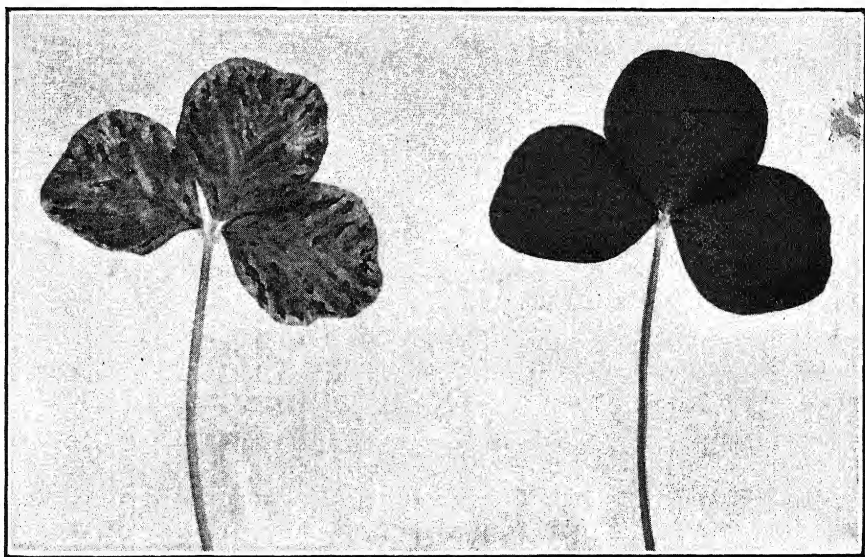
—Photograph by J. A. Carlile.

FIG. 1. *Vicia faba* leaves: left, from plant infected by pea virus 2; right, from healthy plant.



—Photograph by J. A. Carlile.

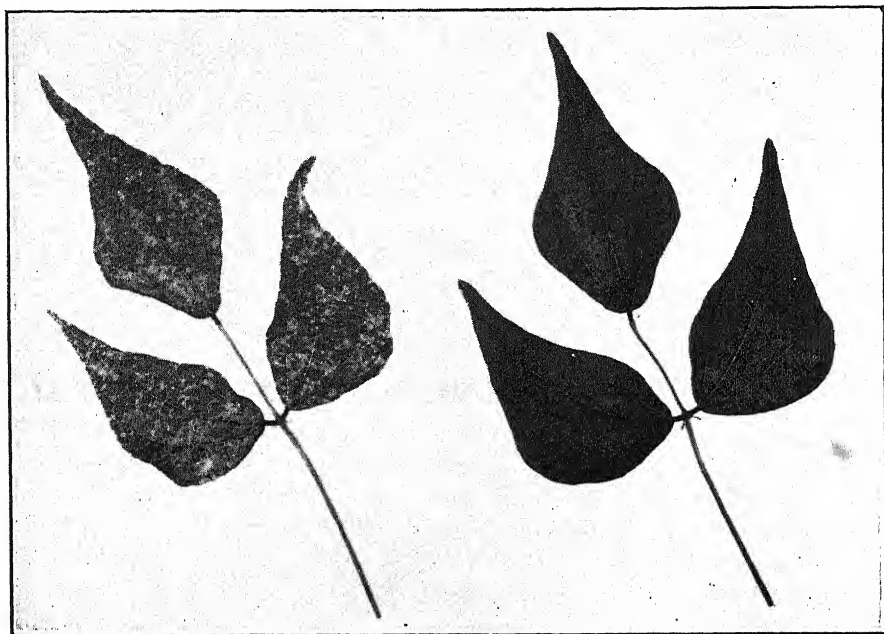
FIG. 2. Alderman pea leaves: left, from plant infected by pea virus 2; right, from healthy plant.



—Photograph by J. A. Carlile.

FIG. 3. Crimson clover leaves: left, from plant infected by pea virus 2; right, from healthy plant.

on the other hand, is only slightly affected by the disease, though a definite mosaic pattern is produced on the leaves. On white sweet clover the disease causes a very mild mottling of the leaves. When transmitted to Robust bean, first symptoms appear as small yellow spots on expanding leaves. In later stages a mild mottle mosaic develops. The mosaic pattern produced in Green Stringless Refugee bean is shown in figure 4.



—Photograph by J. A. Carlile.

FIG. 4. Green Stringless Refugee bean leaves: left, from plant infected by pea virus 2; right, from healthy plant.

It seems appropriate to discuss here the similarity of pea virus 2 and certain pea viruses described by other workers. The mosaic virus of Doolittle and Jones (1), pea-mosaic viruses 1 and 2 of Zaumeyer and Wade (13), and the red clover mosaic virus of Zaumeyer and Wade (13) resemble pea virus 2 on peas, but they differ in reported host range. The value of using Perfection pea to differentiate certain pea-mosaic viruses was shown by Stubbs (11). Pea virus 2 agrees with the pea virus 2 of Stubbs and the pea virus 3 and bean virus 2 of Pierce (9) in being transmissible to several varieties of peas and not to Perfection pea. It differs from the pea virus 2 of Stubbs and the pea virus 3 of Pierce in reported host range. It closely resembles the bean virus 2 of Pierce (9) in symptoms produced on bean varieties, but appears to differ slightly in symptoms produced on peas and in reported degree of resistance to heat. Whether any of these viruses are identical with pea virus 2 is difficult to decide without actual comparisons of

the viruses under similar conditions. It seems likely, however, that further study may prove that some or all of them belong to a single virus group.

#### EXPERIMENTS

*Thermal Inactivation and Resistance to Aging in vitro.* The properties of resistance to heat and to aging *in vitro* are frequently used as a means of classifying or differentiating plant viruses. For this reason, studies were made to determine these properties for pea virus 2. Results of the studies are given in tables 1 and 2. Pea virus 2 was found to be active after heating to various temperatures up to and including 62° C. for 10 minutes, but was inactivated after heating to 64° C. for 10 minutes. The virus was found to be

TABLE 1.—*Thermal inactivation of pea virus 2*

Temperature (degrees C.)	Plants inoculated	Plants infected
52 .....	10	10
54 .....	10	9
56 .....	10	9
58 .....	10	7
60 .....	10	3
62 .....	20	4
64 .....	10	0

TABLE 2.—*Resistance to aging in vitro by pea virus 2*

Time aged	Plants inoculated	Plants infected
15 minutes .....	5	5
6 hours .....	5	5
24 hours .....	5	5
3 days .....	5	1
4 days .....	5	1
5 days .....	20	0
7 days .....	5	0

active after aging *in vitro* for various periods up to and including 4 days, but was inactivated after 5 days' aging.

*Transmission of Pea Virus 2 by Aphids.* In preliminary experiments to determine the method of transmission of pea virus 2 by aphids, colonies of insects were placed on diseased *Vicia faba* plants in screened cages for periods of 24 hours or more and were then transferred to healthy *V. faba* plants. It was found that the pea, potato, and bean aphids served as vectors. However, when colonies of aphids that had fed on diseased plants were transferred to a succession of healthy plants, it was observed that only the set of plants exposed to aphids immediately after they were removed from diseased plants

became infected. The experiments were then repeated, using intervals of from 1 to several hours, in a manner similar to that employed in determining the length of the incubation period of pea virus 1 in the pea aphid (7). Again, only the first set of healthy plants exposed in a succession became infected, indicating that an incubation period, if present, was extremely short, and that the insects quickly lost the virus when transferred from diseased to healthy plants.

*Time Required for Aphids to Acquire and Transmit the Virus.* After it was established that aphids acquired and transmitted the virus within a period of a few hours, a series of experiments was undertaken in which insects were transferred from healthy to diseased plants and then to a succession of healthy plants at very short intervals.

In one experiment 5 colonies of non-viruliferous pea aphids, each consisting of 30 individuals, were placed for a short time on 5 small healthy plants. This was done in order to be sure whether or not the colonies were free from virus. Each of the colonies was then placed on a diseased *Vicia faba* plant for 15 minutes before being transferred to a succession of 4 healthy plants at intervals of 15 minutes, and finally to a 5th set of plants for 1 hour. In this experiment the 5 plants exposed to the aphids before they were placed on diseased plants remained healthy, showing that the aphids were originally noninfective. Each of the 5 plants exposed to aphids during the first period after removal from the diseased plants became infected. One plant exposed during the 2nd period and one exposed during the 4th period became infected. One plant of the 5th set also became infected. The experiment, therefore, demonstrated that the pea aphid is able to pick up the virus within 15 minutes after first feeding on a diseased plant and to transmit it to healthy plants during a 15-minute feeding period immediately following, and that transmission may occur in later periods but is infrequent.

In a similar experiment with potato aphids, 4 colonies, each consisting of 40 individuals, were removed from tomato plants and placed on 4 small *Vicia faba* plants for 15 minutes. Each colony was then allowed to feed on diseased *V. faba* plants for 15 minutes before being transferred to a succession of 4 healthy plants at intervals of 15 minutes. The 4 healthy plants exposed to aphids before they fed on the diseased plants remained healthy. All 4 of the plants exposed to aphids immediately after removal from diseased plants became infected. One plant exposed during the 2nd period also became infected. The 3rd and 4th sets of plants remained healthy. The experiment demonstrated that the potato aphid, as well as the pea aphid, acquires and transmits pea virus 2 within a total period of 30 minutes. In 3 of the 4 colonies, the virus was lost by the aphids after feeding on healthy plants for a period of 15 minutes. In the 4th colony the virus was lost by the aphids after feeding on healthy plants for 2 successive 15-minute periods.



In the same way, 8 colonies of bean aphids, each consisting of 30 adults or large nymphs, were fed first on healthy *Vicia faba* plants and then on diseased *V. faba* plants for 15 minutes before being transferred to a succession of 4 healthy plants at intervals of 15 minutes. The 8 healthy plants exposed to aphids before they fed on diseased plants remained healthy. Five colonies infected plants on which they were placed immediately after removal from the diseased plants. Three of these infected plants during the 2nd period and 1 during the 4th period. The experiment demonstrated that the bean aphid, as well as the pea and potato aphids, can acquire and transmit pea virus 2 within a period of 30 minutes.

To determine whether either the pea, potato, or bean aphids would transmit the virus after a period of feeding shorter than 15 minutes, tests were made employing 5-minute intervals. Five colonies of each of the 3 species were tested. Again, each colony consisted of 30 individuals. Prior to the experiment, the colonies were held without access to food for a time, since it was thought they might feed more readily after such isolation. The colonies of bean aphids and 2 colonies of pea aphids were held without food for about 3 hours, while the remaining colonies were isolated from food overnight. Each colony was then fed on a healthy *Vicia faba* plant for 15 minutes before being placed on a diseased plant. This was done in order to determine whether or not the colonies were free from virus. The insects remained on the diseased plants for 5 minutes. After removal, each colony was transferred to a succession of 2 healthy plants at 5-minute intervals and then to a healthy plant for 15 minutes. The results obtained in this experiment are shown in table 3. The 15 plants exposed before the aphids were fed on diseased plants remained healthy, showing that the colonies were originally noninfective. All of the healthy plants in the successions exposed to colonies of the pea

TABLE 3.—Infections obtained with colonies of aphids when transferred from healthy to diseased plants for a period of 5 minutes and then to a succession of healthy plants. Each colony consisted of 30 aphids

Period	Length of exposure	Condition of plants when exposed	Macrosiphum pisi					Macrosiphum gei					Aphis rumicis				
			1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
1st .....	15 min.	Healthy	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
2nd .....	5 "	Diseased	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
3rd .....	5 "	Healthy	+	+	+	+	+	+	+	—	+	+	—	—	+	+	+
4th .....	5 "	Healthy	+	+	+	+	+	+	—	+	+	+	—	—	—	—	—
5th .....	15 "	Healthy	+	+	+	+	+	+	+	+	+	+	—	—	—	—	+

+ = Plant became infected.

— = Plant remained healthy.

aphid after they had fed on diseased plants became diseased. Of the 5 plants exposed to potato aphid colonies for 5 minutes immediately after the aphids were removed from diseased plants, 4 became diseased. Four plants exposed during the 2nd 5-minute period, and 5 exposed for 15 minutes in the 3rd period became diseased. Likewise, 3 infections were obtained in the 5 plants exposed to bean aphids during the 5-minute period immediately following removal from diseased plants, and 1 infection was obtained in the 5 plants exposed during 15 minutes in the 3rd period. The experiments, therefore, demonstrate that the pea aphid, the potato aphid, and the bean aphid are able to pick up the virus within 5 minutes after first feeding on diseased plants and are able to transmit it to healthy plants during a 5-minute feeding period immediately following. The experiments also demonstrate that the colonies continue to be infective after 2 feeding periods of 5 minutes each on healthy plants.

Some aphids do not begin to feed immediately after being transferred from one plant to another. A few continue to move about on the leaves or stems throughout a short period. It is believed that this accounts in part for the fact that there is no apparent loss of virus by pea and potato aphids after 2 successive transfers to healthy plants at 5-minute intervals. The small number of infections obtained by means of the bean aphid after short feeding periods is believed to be due to the difficulty in transferring the colonies quickly. Pea aphids drop from a plant at a slight touch of the brush and are quickly transferred to another plant without any apparent injury. Potato aphids, likewise, may be transferred in a short time, though they are more difficult to handle than pea aphids and a few appear to be injured at each transfer. Bean aphids, on the other hand, stick tightly to the plants. To transfer them in a short period of time requires forcible brushing that causes considerable injury.

In transferring colonies of aphids by means of camel-hair brushes, the possibility was considered that infection might be due to contamination from the brushes. For this reason, in several experiments the brushes used to transfer aphids from diseased plants were rubbed over the leaves of healthy plants, but in no case was infection obtained by this means.

*Loss of Virus by Aphids when Removed from Diseased Plants and Held for 1 Hour on Healthy Plants.* In the previous experiments it was observed that, while some of the colonies lost the virus when held for 15 minutes on healthy plants, others produced infection in plants exposed during subsequent periods. An experiment was, therefore, made to determine whether or not the virus would be retained in aphid colonies held continuously on a healthy plant for 1 hour. In this experiment 2 colonies of the pea aphid, each consisting of 45 individuals, and 5 colonies of the potato aphid, each consisting of 30 individuals, were fed on diseased *Vicia faba* plants for 18 hours. On

removal from the diseased plants each colony was transferred to a healthy plant and allowed to feed for 1 hour. They were then transferred to a 2nd set of healthy plants and held for a period of 18 hours. In a similar way, bean aphids were fed on diseased *V. faba* plants for 1 hour. Five colonies, each consisting of 30 individuals, were then placed on a set of 5 healthy *V. faba* plants for a period of 1 hour before being transferred to a 2nd set of 5 healthy plants on which they were also held for 1 hour. One of the plants of the first set exposed to the pea aphid, 5 of the first set exposed to the potato aphid, and 2 of the first set exposed to the bean aphid became diseased. None of the plants in the 2nd set, exposed to either one of the 3 species of aphids, became diseased. The results indicate that all 3 species of aphids lose the virus when allowed to feed continuously on healthy plants for 1 hour.

*Retention of Virus in Aphids Held without Access to Food Plants.* Since it was found that aphids lost the virus during a short period of feeding on healthy plants, it seemed of interest to determine how long they would retain it when held without access to food. For this purpose pea aphids, potato aphids, and bean aphids were fed for at least 1 hour on diseased plants. Some were then transferred immediately to healthy plants to be sure that the insects had acquired the virus. Others were removed from the diseased plants and placed in containers where they were held without access to food for periods of 1 to 48 hours before being transferred to healthy plants. Several types of containers were tried in these tests, the most satisfactory being clay pots with bandage gauze tied over the tops. In clay pots pea and potato aphids survived for 24 hours in good condition, but a good many were dead at the end of 48 hours. Colonies of the bean aphid were noticeably affected by isolation without food for 18 hours. Colonies of 30 individuals were placed on each of the *Vicia faba* plants exposed in these tests. The results obtained are shown in table 4. Colonies of the pea, potato, and bean aphids, held without access to food for 1 hour, infected plants in about the same proportion as colonies transferred immediately from diseased to healthy plants. The results with colonies held for more than 1 hour varied somewhat with the different species of aphids. The infections obtained demonstrate that the bean aphid may retain the virus for at least 5 hours, the pea aphid for at least 8 hours, and the potato aphid for 24 hours, when the insects are held without access to food plants after removal from diseased plants.

*Tests for a Long Incubation Period of the Virus in Aphids.* The experiments previously described have shown that pea virus 2 is transmitted by aphids within a few minutes after first feeding on diseased plants. This type of transmission appears to be merely the transfer of virus in the act of feeding and does not involve an incubation period in the insect. The possibility remained, however, that aphids might again become infective after

TABLE 4.—Infections obtained with colonies of aphids which, after removal from diseased plants, were held for a period of time without access to food plants and then placed on healthy plants. Each plant was exposed to a colony of 30 aphids

Length of period without access to plants	Macrosiphum pisi		Macrosiphum gei		Aphis rumicis	
	Plants exposed	Plants infected	Plants exposed	Plants infected	Plants exposed	Plants infected
15 minutes .....	27	17	25	25	15	12
1 hr. ....	5	3	5	3	5	4
2 hrs. ....	"	2	"	3	"	1
3 " ....	"	2	"	3	"	2
4 " ....	"	1	"	2	"	2
5 " ....	"	1	"	2	"	3
6 " ....	"	2	"	1	"	0
7 " ....	"	0	"	2	"	0
8 " ....	"	2	"	1	"	0
10 " ....	"	0	"	0	"	0
12 " ....	"	0	"	2	"	0
18 " ....	"	0	.....	.....	"	0
24 " ....	"	0	8	1	.....	.....
48 " ....	"	0	5	0	.....	.....

incubating the virus for a long period of time. To settle this point, infective colonies of the pea, potato, and bean aphids were placed on healthy plants and held for a total period of 14 days. Several times during this period the colonies were transferred to fresh plants and at each transfer newborn nymphs were destroyed.

In one test, 300 pea aphids were fed for 24 hours on diseased *Vicia faba* plants. Half of them were placed on healthy plants immediately and half were held without access to food for 12 hours. Both lots were then transferred to 4 sets of healthy plants at intervals of several days. Ninety-seven aphids were alive at the end of 14 days. Two of the plants exposed immediately after the aphids were removed from the diseased plants became diseased. No mosaic disease developed in any of the plants subsequently exposed to the aphids. It is concluded that no incubation period of pea virus 2 occurs in the pea aphid.

In a test with potato aphids, nymphs were fed for 24 hours on diseased *Vicia faba* plants. Thirty were then transferred to each of 5 healthy *V. faba* plants and several hundred were placed on small tomato plants. At the end of 5 days, 150 aphids were removed from the tomato plants and placed on 5 healthy *V. faba* plants. The remaining aphids were transferred to other tomato plants. After 12 days, 120 adult potato aphids remaining on the tomato plants were transferred to 5 healthy *V. faba* plants and held for a period of 2 days. The 5 *V. faba* plants exposed to nymphs of the potato aphid

immediately after removal from diseased plants became infected. The 5 *V. faba* plants exposed to aphids that had fed on tomato plants for 5 days and the 5 *V. faba* plants exposed to aphids that had fed on tomato plants for 12 days remained healthy. From this it is concluded that no incubation period of pea virus 2 occurs in potato aphids when fed on diseased plants and then held on healthy plants for a total period of 14 days.

In a test with the bean aphid, 500 large nymphs and adults were fed for 2 hours on diseased plants and then placed on 5 healthy plants for a period of 24 hours. Subsequently the aphids were transferred to fresh plants at intervals of several days, the colonies being held for a total period of 14 days. Three hundred and twenty aphids were transferred to the final set of healthy plants. Three of the plants exposed to aphids immediately after removal from diseased plants became infected, but no infections were obtained in any of the plants exposed at subsequent transfers. It is, therefore, concluded that no incubation of the virus occurs in the bean aphid.

The experiments with the pea, potato, and bean aphids demonstrate that these insects are infective only for a short period immediately after they have fed on diseased plants, and that they do not become infective again unless they are again allowed to feed on diseased plants.

*Transmission of Pea Virus 2 by Single Insects.* A factor of considerable importance in the spread of virus diseases is the effectiveness of single individuals as carriers. The studies on transmission by means of colonies gave no indication as to this ability in the pea, potato, or bean aphids. Infection produced by colonies might be the result of many small injections of virus rather than of inoculation by a few individual carriers within the colony. Experiments, therefore, were undertaken to determine the effectiveness of single insects in this regard. In some preliminary trials, 75 *Vicia faba* plants were exposed to single aphids that had fed on a diseased plant, but none of them became infected. The reason for the failure to obtain infection in these early trials is not known. In a subsequent experiment, 200 adult pea aphids were fed for 1 hour on diseased *V. faba* plants. Thirty aphids were then placed on each of 5 healthy plants, while single aphids were placed on each of 50 small healthy plants. Of the 5 plants exposed to colonies, all became infected. Of the 50 plants exposed to single insects, 10 became diseased. Nine of the 50 single aphids were winged and one of these proved to be a carrier. This experiment demonstrated, therefore, that single adult pea aphids, both alate and apterous, are capable of transmitting pea virus 2. It also demonstrated that a much smaller proportion of plants are infected when exposed to single insects than are infected when exposed to colonies.

In a comparable test, 200 pea aphid nymphs were placed on diseased *Vicia faba* plants for 1 hour. Five colonies of 30 nymphs each were then transferred to healthy plants to serve as a control, while 1 nymph was trans-

ferred to each of 50 healthy plants. Of the 5 plants exposed to colonies, all became diseased. Four of the 50 plants exposed to single nymphs became diseased. This experiment, therefore, demonstrated the ability of single nymphs to transmit pea virus 2.

To test the ability of individual potato aphids to transmit pea virus 2, 25 *Vicia faba* plants were exposed to adults that had fed for 1 hour on diseased plants. Five of these became infected. In similar tests with the bean aphid, 20 plants were exposed to individual adults that had fed on diseased plants for 1 hour, and of these 3 became infected. The tests show that pea virus 2 is transmissible by individual potato and bean aphids, as well as by individual pea aphids.

#### DISCUSSION

The experiments presented in this paper show that the pea aphid, the potato aphid, and the bean aphid acquire and are able to transmit pea virus 2 within a period of 10 minutes after first feeding on diseased plants. The experiments also show that the aphids are infective only for a short period immediately after they have fed on diseased plants, and that they do not again become infective unless again allowed to feed on diseased plants. Transmission of the disease in this manner would appear to represent merely a mechanical transfer of the virus as one constituent of plant extract, regardless of whether the virus is carried from diseased to healthy plants on the mouth parts or is imbibed and transmitted through the bodies of the insects.

The method of transmission of pea virus 2, therefore, is distinctly different from that shown in the transmission of pea virus 1 by the pea and potato aphids (7, 8). The latter is transmitted only after an incubation period of from 12 to 24 hours in the aphids, and is retained during the life of the insects. The reason for this difference in method of transmission is not known.

#### SUMMARY

A mosaic disease caused by a virus designated as pea virus 2 was transmitted by mechanical methods from *Vicia faba* to garden pea, sweet pea, field pea, red clover, crimson clover, and several varieties of beans. From each of these hosts it was transmitted back to *V. faba*. No infection was obtained in Wisconsin Resistant Perfection pea. The virus was found to be active after heating to a temperature of 62° C. for 10 minutes, but was inactivated when heated to 64° for 10 minutes. The virus was active after aging *in vitro* for 4 days, but was inactivated after 5 days' aging.

The virus was transmitted by both nymphs and adults and by single insects of each of 3 species of aphids, the pea aphid, the potato aphid, and the bean aphid. It was found that colonies of each of these aphids are able

to acquire the virus during a feeding period of 5 minutes on a diseased plant and are able to transmit it to a healthy plant during a 5-minute period immediately following. Some colonies lost the virus during a period of 15 minutes on healthy plants. Retention of the virus for more than 1 hour was not demonstrated in any colonies allowed to feed continuously on healthy plants for 1 hour. When held without access to food, the bean aphid was shown to retain the virus for 5 hours, the pea aphid for 8 hours, and the potato aphid in one instance for 24 hours. No incubation period of the virus was observed in colonies of the pea, potato, or bean aphids that were fed for 1 day on diseased plants and were then transferred to a succession of healthy plants for a total period of 14 days.

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# BUNCH DISEASE OF PECANS

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## INTRODUCTION

A disease of pecan trees (*Hicoria pecan*) named "bunch" on account of its characteristic symptoms, was first determined in the spring of 1932 on trees growing in the Red River Valley near Shreveport, Louisiana. Previous to this time the bunch disease had been confused with pecan rosette, a nutritional disease, and was not identified as a distinct disease until the control of rosette by the use of zinc sulphate was perfected (1, 3).

## SYMPTOMS

A characteristic and distinguishing symptom of the bunch disease is the brooming of branches and shoots. This symptom may appear on small and on large lateral branches and on sucker growth, extending throughout the entire length of large branches. In advanced cases, clusters of willowy sprouts may take origin direct from the large main limbs. Many of the axillary buds of the current year's growth produce lateral shoots the same season the main shoot is formed. Apparently these axillary buds develop into shoots soon after the buds are formed since the resultant lateral shoots are frequently as long as is the primary shoot. Furthermore, in some cases the lateral buds on these lateral shoots are also forced into growth. In advanced stages of the disease the new shoots are short and slender and the buds are weak and in many cases fail to develop. This multiplicity of branching gives the diseased portions of the tree a witches'-broom or "bunching" effect (Fig. 1). Because of this characteristic forcing of the lateral buds into shoots resulting in a bunching of the shoots, the writer chose "bunch" as an appropriate and descriptive name for the disease.

The color of the diseased leaves varies from dark green to chlorotic, the terminal leaflets are subnormal in length and usually all of the leaflets are abnormally thin and above normal proportions in breadth. The leaflets on diseased shoots are frequently wavy or twisted and give an appearance of being slightly wilted (Fig. 2). In some instances, especially where the growth has been forced, the leaflets are twice as large as healthy leaflets on normal but forced shoots. The diseased leaflets do not persist until normal leaf fall but begin to absciss in late summer or early in the fall season. This abscission begins on the lower leaves of the diseased shoot and pro-

<sup>1</sup> The writer wishes to express his appreciation for their critical review of this manuscript to H. L. Crane, Lee M. Hutchins, and J. B. Demaree, Bureau of Plant Industry, U. S. Department of Agriculture.



gresses upward as the season advances. In many cases the leaf rachises do not absciss until late into the fall, or even winter.

In most instances bunch is more easily recognized in the early than in the advanced stages. In the early stages the disease is confined to a branch, or small portion of the tree, while in the advanced stage, it covers the entire tree (Fig. 3).

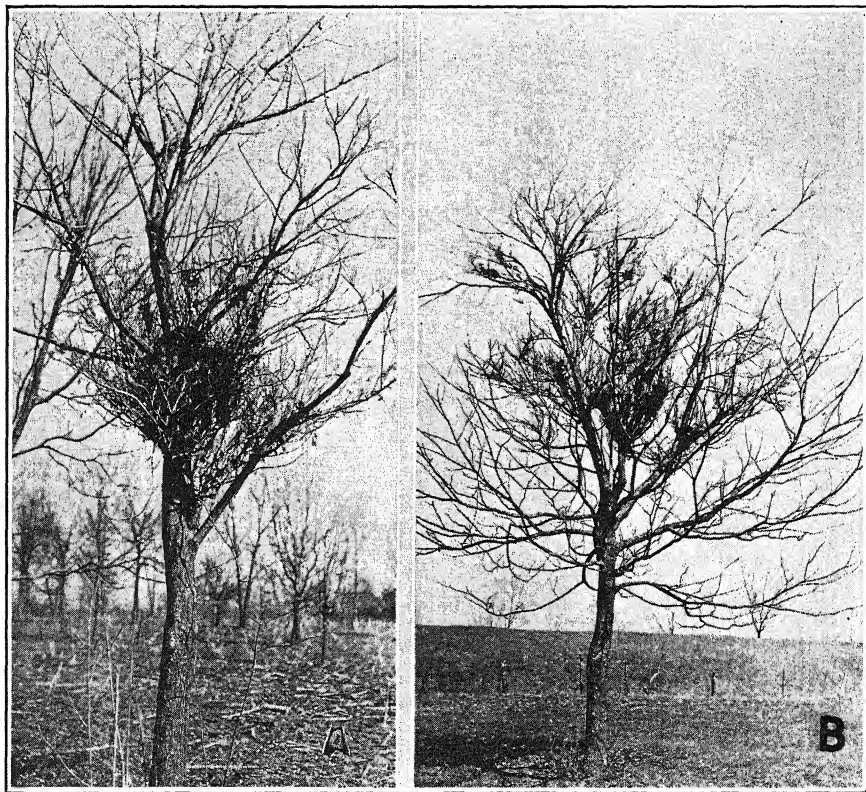


FIG. 1. Pecan trees affected with bunch disease in the Red River bottoms, near Texarkana, Tex. A. Native seedling. B. Schley variety in an orchard.

The shoots and small branches in the advanced stages of the disease are usually discolored, brittle and often die back. Death of large limbs of the trees eventually occurs.

It does not appear that the disease attacks a specific part of the tree, since it may appear in the lower, central or topmost portions of the tree and then spread to the adjacent branches until the entire tree becomes diseased. While we have no record of trees actually dying from the disease, there are several instances where the trees were so severely diseased that the growers

destroyed them. Water hickory trees (*Hicoria aquatica*) growing along the bayou banks near Shreveport, Louisiana, affected with the bunch disease, have been found dead, but whether the bunch disease killed these trees or whether it was of secondary importance was not established. Excepting for the bunch disease these trees appeared normal.

Nut production is greatly reduced on severely diseased trees; however, some marketable nuts may be borne, though many of them are undersized and occasionally poorly filled. This production of marketable nuts is another characteristic of the disease distinguishing it from others with which it might be confused.

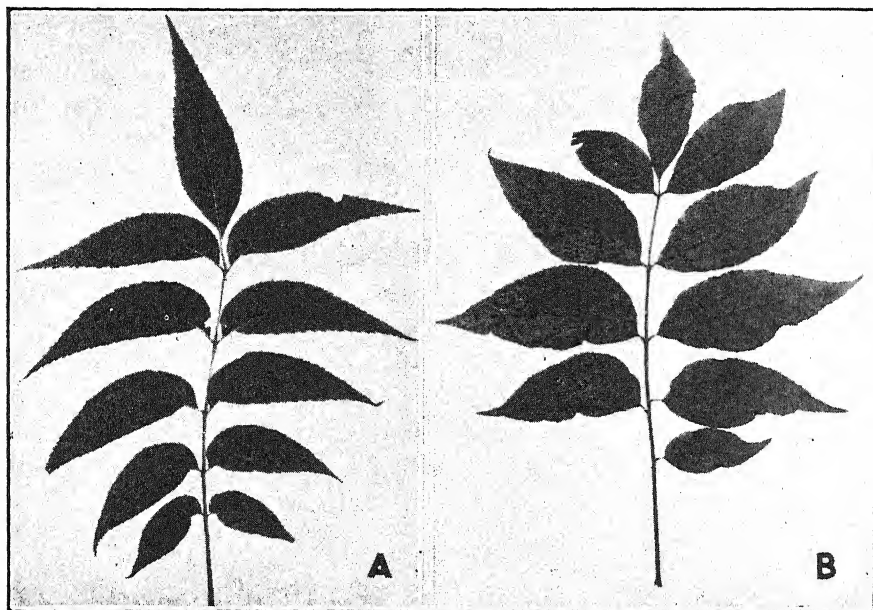


FIG. 2. Leaves of Schley pecan. A. Normal. B. Affected with bunch disease.

Another important and distinguishing symptom of the bunch disease is the early foliation of the diseased branches and shoots in spring. Foliation of diseased portions of the tree may be ten days to two weeks in advance of the healthy parts of the tree (Fig. 4). Hence, observations made just prior to normal foliation readily discloses the disease in trees where it might be overlooked later in the season.

#### SIMILARITY OF SYMPTOMS OF BUNCH DISEASE TO SIMILAR DISEASES OF OTHER SPECIES

Bunch disease has some similarity to pecan rosette, especially chlorosis, but the chlorosis on rosetted leaflets is confined to the spaces between the

veins, giving the leaflets a striped appearance while the chlorosis on some leaflets affected with the bunch disease is more uniform over the entire leaflets. On the other hand, some of the leaflets affected with the bunch disease show little, or no signs of chlorosis. Furthermore, rosetted leaves are small, narrow, crinkled, thick, harsh, and brittle, while those affected with bunch disease are thin, broad, wavy, and flexible. Some brooming occurs on rosetted trees as a result of the forcing of lateral buds caused by

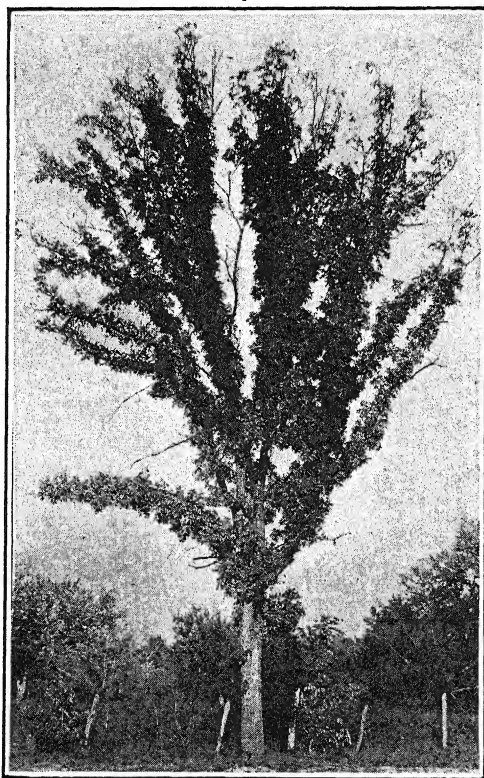


FIG. 3. Native pecan tree growing on upland soil near Shreveport, Louisiana. Reliable information indicates that when this photograph was made this tree had been affected by the bunch disease for 10 years; all limbs and branches were diseased.

the dying back of the primary shoots, but dying back of the shoots affected with bunch disease occurs only in the more advanced stages of the disease. Unfortunately, trees may be affected with both diseases at the same time, when diagnosis becomes difficult. In the advanced stages of both diseases the dying back of shoots, branches, and large limbs often occurs.

Bunch disease of the pecan is quite similar in appearance to the witches' broom, a virus disease, of black locust, *Robinia pseudacacia* (4). Further-

more, it has at least one characteristic of the phony peach disease (6), in that phony trees usually foliate several days in advance of normal trees. Pecan trees affected with bunch disease do likewise. In this respect bunch is also similar to peach yellows (5, pp. 267-268).

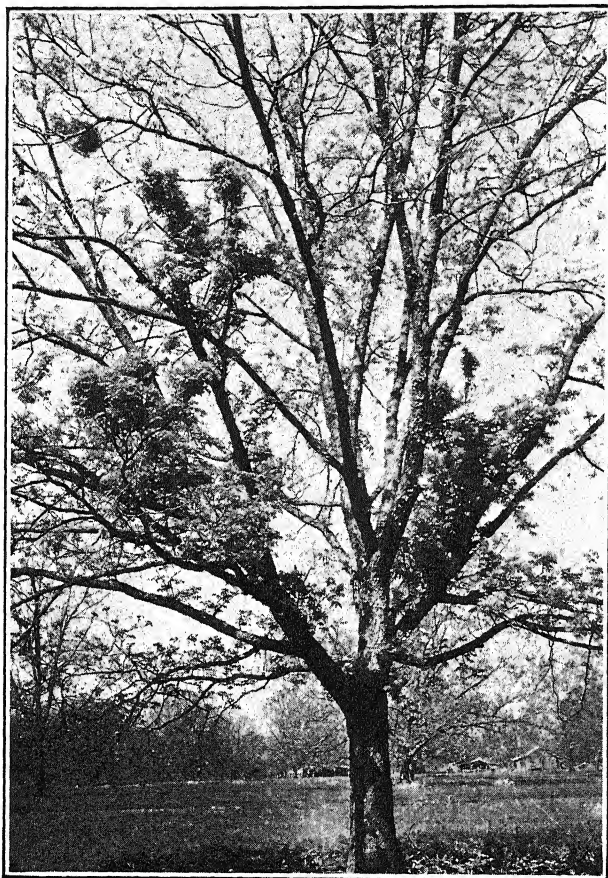


FIG. 4. Diseased portions of Schley pecan tree affected by the bunch disease, which foliate before normal parts.

Trees badly affected with "little leaf" disease of pecans bear no nuts (2), while trees affected with bunch disease may bear some marketable nuts. Trees affected with the rosette disease may bear some nuts but they are usually abnormally small and poorly filled. Oftentimes they fail to grow much larger than the pistillate flower, especially on the badly diseased shoot.

#### DISTRIBUTION

Special scouting trips have not been made to determine the full range of the bunch disease. The geographical range known at present extends

east into Mississippi along the Mississippi River; west almost to Austin, Texas; north 50 miles beyond Wewoka, Oklahoma, and south almost to Alexandria, Louisiana.

Bunch disease is mostly confined to the alluvial river bottom soils. Perhaps the appearance of the disease on these particular soils may be explained by the fact that, in all probability, bunch first attacked the native seedling or wild pecans (*H. pecan*) and water hickories (*H. aquatica*). The native or wild pecans and water hickories are indigenous to the river-bottom section of the states where this disease has been observed.

#### SUSCEPTIBLE VARIETIES

The pecan varieties most susceptible to the bunch disease are the Mahan, and the Schley, followed by the Burkett, Mobile, Success, Centennial, Pabst, Van Deman, Russell, and the Moneymaker. Still other varieties may be found to be susceptible to the disease. The Stuart is apparently highly resistant or it is a symptomless carrier of the disease. Several diseased native seedling trees have been top-worked to Stuart and the scions have now remained healthy for about seven years.

To what extent native seedling pecan trees are resistant or susceptible is not known. In one planted seedling orchard about 60 miles south of Shreveport, Louisiana, where the trees are 35 years old, 48 out of 50 trees are slightly to severely diseased.

#### EXPERIMENTS TO DETERMINE THE CAUSE OF BUNCH DISEASE

Some fungi and bacteria were isolated from diseased parts but all were regarded as saprophytes.

The first experiments to determine the cause of the bunch disease were begun in August, 1932. Diseased leaves and juices from diseased leaves of the Schley variety were used to inoculate healthy Schley leaves, but the results of the inoculations were all negative. The trees were destroyed in 1933 and records were not available. Later, experiments were made to determine the transmissibility of the disease by inoculation with juices from diseased leaves through injecting such material into holes bored into the limbs of healthy trees. Numbers of the black pecan aphid (*Melanocallis caryaefoliae* Fitch) were also transferred from diseased to normal leaves in an effort to determine whether they were vectors. In all cases the results were negative.

In the early spring of 1933 twenty trees of the Schley variety, showing signs of both the bunch disease and pecan rosette, were sprayed three times with a 1-50 zinc sulphate solution. This spray failed to prevent or overcome the bunch disease, but prevented the development of rosette and stimulated new growth. This indicated that bunch was not closely related to rosette, a zinc-deficiency disease.

Additional experiments on the possible infectious nature of the disease were begun in the early spring of 1934. These experiments were conducted in orchards where the bunch disease was present in order to avoid accidental dissemination to localities not already infected, in case the disease proved to be infectious. Scions from diseased Schley trees, to be used for grafting onto healthy trees, were classified as follows: (1) Wood badly diseased, brittle, discolored, buds slender and poorly developed; (2) wood of normal color, buds slender and poorly developed; (3) scions normal in appearance except for the presence of axillary buds making the "bunching" effect, which the writer considers to be the first stage of the bunch disease; (4) scions apparently free of the bunch disease. The scions were grafted onto *H. pecan*, using the Schley, a variety very susceptible to the disease, and the Stuart, a variety very resistant, and onto other species of hickory, probably *H. glabra* and *H. alba*. Six scions of each class were used in each of the series of experiments.

Growth from diseased scions (Classes 1, 2, and 3) grafted onto the Schley pecan variety was normal for about 60 days, then began showing symptoms of the bunch disease. The growth from scions in Class 4 remained healthy. Growth from diseased scions grafted onto the Stuart pecan variety and other species of hickory (Classes 1, 2, and 3) was apparently healthy and free of the bunch disease. In June, 1936, the only change that had taken place since July, 1934, was the progress that the disease had made in the first three classes on the Schley variety. The growth from the scions was severely bunched, and the disease had spread below the graft unions at least two feet and perhaps in some instances five to six feet. The growth from those scions on the Stuart pecan variety and other species of hickory, *glabra* and *alba*, had not changed since 1934 but remained normal and healthy. The growth from those scions in Class 4 (check) also remained normal and healthy.

Extreme difficulty was encountered in getting the scions of the first two classes to grow. Not over 20 per cent of Class 1 nor 50 per cent of Class 2 grew, even under what was considered to be ideal growing conditions. This was in contrast to about 80 per cent of Classes 3 and 4 that grew.

From one to three diseased limbs were cut from each of twelve otherwise apparently healthy Schley trees in June, 1934, in orchards located near Shreveport, Louisiana. Ten of these trees were located in a 200-acre orchard where the disease was quite prevalent, while two were the only trees known to be affected in one 500-acre orchard. These limbs were removed 5 to 6 feet below the visible signs of bunch disease. In April, 1935, the disease had reappeared in some of the trees, while observations made in June, 1936, disclosed that the disease had reappeared in each of the ten trees in the orchard where the bunch disease was prevalent. In some instances

bunch disease reappeared near the wounds, while in others it reappeared in different parts of the trees. The disease had not reappeared in the two trees, after three growing seasons, located in an orchard where they were the only ones known to be affected by the bunch disease.

#### DISCUSSION

Experimental data and observations indicate that the bunch disease of pecans is infectious. That it may be a virus disease is suggested from the facts that (1) no visible parasitic organism has been identified in diseased material; (2) no parasitic fungus or bacterium has been recovered in the usual laboratory cultures on artificial media; and (3) diseased scions grafted onto normal susceptible plants transmit the disease.

The disease apparently spreads rather slowly. In two instances, (1) an orchard located near an abandoned nursery composed of trees of the Schley variety showed heavy infection of the Schley variety on that side nearest the nursery, with the amount of infection decreasing as the distance from the nursery increased. The nursery was near diseased wild water hickories, *H. aquatica*. (2) From a heavily affected seedling grove the disease had spread to some of the surrounding trees, especially the Schley and Mobile varieties.

There were two characteristics brought out during the course of experiments indicating that the bunch disease might be more easily controlled or eliminated than other diseases of this nature. (1) Diseased Schley scions grow normally when placed on healthy Stuart trees. Since the diseased scions, instead of growing normally through the incubation period of about 60 days and then becoming diseased, were making normal growth at the end of the third season, there is an indication that the Stuart variety is capable of inducing an apparent recovery from the disease. Resistance of hosts to virus diseases is a well-known fact with a great many plants, but the writer has been unable to learn of resistant stocks overcoming the disease in affected scions. If the Stuart stock will produce immunity in susceptible scions, as the experiments indicate that it will, this will be of considerable value and aid in controlling the bunch disease. When growers desire varieties other than Stuart, they might practice "double working" trees, similar to the method used by apple growers in eliminating collar rot. However, Stuart scions grafted onto trees affected by bunch disease do not overcome the disease in the stock. (2) Elimination of the bunch disease by the removal of diseased limbs and twigs from otherwise normal trees. The experimental data indicate that this may be done if only a few trees in an isolated orchard are affected by the disease. Preventing the spread of a virus disease in a plant by removing the diseased parts is not common. As the above experimental data indicate, after the disease has been firmly



established in an orchard, removing diseased limbs would be of little or no value in controlling the disease.

#### SUMMARY

Bunch disease of pecans, which in the spring of 1932 was definitely determined to be new to that species, was found in the Red River Valley of Louisiana, near Shreveport. Characteristic and distinguishing symptoms of the bunch disease are the brooming of branches and shoots, early foliation of diseased branches in the spring, chlorotic, thin, broad, wavy, and flexible leaves, and in later stages dying back of the branches. This bunch disease has certain symptoms similar to rosette and in some instances it is difficult to distinguish between the two. It also has certain characteristics similar to other diseases, namely, little-leaf of pecans; two important diseases of peaches, phony peach and peach yellows; and witches' broom of black locust.

Bunch disease is known to be present in the following states: Louisiana, Mississippi, Oklahoma, and Texas.

The disease was successfully transmitted by grafting diseased Schley scions onto healthy Schley stocks.

The Mahan and the Schley are among the most susceptible varieties, while the Stuart variety is highly resistant.

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## A BACTERIAL BLIGHT OF IRIS

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Observations since 1934 on iris in gardens about Ithaca, New York, have revealed considerable leaf blight, which could not be attributed entirely to the heterosporium leaf spot, caused by *Didymellina macrospora* Kleb., nor to the bacterial soft rot due to *Erwinia carotovora* (Jones) Com. S.A.B. In many instances where a blight occurred, the symptoms were distinct from those of these two diseases. The new blight of iris appeared to be due to a bacterial infection, but it differed distinctly from the soft rot, which is not primarily a disease of the foliage. Any leaf blight in connection with the soft rot is the result of the destruction of the lower parts of the plant. The blight under discussion is a disease of the leaf blade only, and a rot of the rhizomes has not been observed. Furthermore, the disease does not necessarily appear at the base first and progress upward, but may begin as a small spot or spots at any place on the leaf. The blight also differs distinctly from the heterosporium leaf spot, in that this disease is a typical leaf spot and has a fungus associated with it.

The symptoms of the disease, as observed during the summers of 1934 and 1935 at Ithaca, are as follows. The lesions may become evident first as small pale areas in the leaves, and are not localized in any special portion. In the early stages these areas may not be observed unless sought for, and are seen clearly only by holding the leaf to the light. In certain instances *Liesegang* rings are noticeable and the leaves appear to be affected with a mosaic. The light areas in figure 1 picture this stage, but here the symptoms are brought out with special distinctness because the specimens were photographed by transmitted light.

These primary symptoms may exist for a week or longer, while the lesion gradually expands, especially longitudinally, in the leaf. Eventually the diseased tissue breaks down into partially water-soaked spots, which dry down to a brown necrotic lesion. Occasionally the water-soaked appearance is absent. Under moist conditions a sticky exudate is noticeable over the lesion, and when dry may be removed from the leaf as a fine scale. The edges of the leaf blade appear to be more susceptible to infection than the central part, and it is this portion that first succumbs to the disease. In severe cases, which are frequent, the entire leaf dies.

When a leaf showing the early stages of the disease is removed from the plant and the cut end placed in water, the collapse of the tissue takes place very rapidly.

The leaf blight of iris probably is not a disease of recent origin, but, due to its similarity to the soft rot, it has been overlooked. Its distribution at present is unknown, but apparently is of wide-spread occurrence and certainly not limited to the section about Ithaca. H. H. Whetzel collected specimens of diseased iris leaves at Crawfordsville, Indiana in 1928. Photographs of these specimens, made at that time, show typical symptoms of this

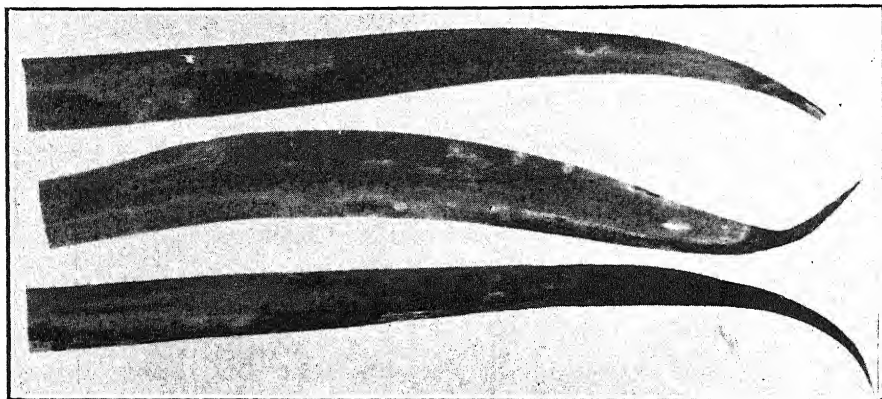


FIG. 1. The bacterial leaf blight of iris caused by *Phytomonas tardicrescens*.

disease. Iris leaves sent to C. E. F. Guterman by O. C. Boyd from Massachusetts in 1931, probably were affected by this disease; and in a conversation with the writer, H. T. Cook told of a leaf blight of iris in Virginia, which was possibly the same disease.

The leaf blight of iris seems to be favored by the higher temperatures of midsummer. During the last two seasons the blight has not appeared early at Ithaca. Careful examination of diseased leaves during May and early June show that at this time *Erwinia caratovora* or other soft-rot organisms are present, but not until the middle of June does one begin to find the blight, even in limited amounts. Later, it may become abundant.

A moist season probably is most favorable for the development and spread of the disease. One planting of iris at Ithaca showed a large amount of infection during the summer of 1935. The same planting showed a small amount on June 15 of the following year. The 1936 season was exceptionally dry, and by August 1, with rainfall approximately one-third below normal for June and July, the disease had not spread and was difficult to find. Temperatures during the dry spell were at times exceedingly high, reaching a maximum of 103° F.

Observations have shown the following species to be susceptible: *Iris germanica* L., *I. florentina* L., and *I. fulva* Ker., but a careful study of the

species range in the genus *Iris* has not been made. Varietal susceptibility within these species may exist, but no investigations have been conducted on this phase of the problem.

A microscopic examination of the lesions from the earliest stages reveals the presence of myriads of bacteria. Such an examination is best effected by making a congo-red smear of the diseased tissue. The bacteria are very small, much smaller than the cells of *Erwinia caratovora*, and a direct comparison of the soft rot pathogen with the blight pathogen is not necessary to discern the difference. Examination of lesions in early stages will show that the bacteria are located primarily in the xylem vessels, but in later stages of the disease they may be found in the surrounding tissue. The bacteria are not limited entirely to the obviously diseased tissue, but may extend in the vascular system far out into the green and healthy appearing portions of the leaf. In certain cases they have been found from 7 to 8 cm. above the edge of the lesions. It appears from these observations that the pathogen is primarily a vascular parasite and one that does not produce substances very toxic to the vascular tissue.

#### ISOLATION OF ORGANISM

The bacteria associated with the leaf blight were isolated at first with some difficulty. When beef-extract peptone agar is used as the medium and dilution plates are made from the leaf lesions, small circular, yellow, slow-growing colonies may appear in 4 or 5 days. If 0.5 per cent of dextrose is added to the above medium, the time period for the bacteria to appear is shortened and the colonies are somewhat larger in size. The same type of colony appeared predominately in all isolations. When the organism is grown for some weeks in culture it adapts itself to its environment more readily and, in a transfer, may appear in 24 to 36 hours.

#### PATHOGENICITY

Inoculation experiments were made in the greenhouse to test the pathogenicity of the isolated bacteria. Healthy iris plants were removed from the garden and placed in pots; and in none of the experiments did the checks develop the disease, whereas symptoms of it appeared in those plants inoculated with the above bacteria.

Through many inoculation experiments, conducted under various conditions, two factors appeared necessary for good infection. First, it was essential that the leaf be injured. In other words, stomatal invasion and infection did not take place, which is in accordance with results obtained in inoculation experiments with some of the other bacterial vascular parasites.

And second, inoculated plants had to be kept in a humid atmosphere under bell glasses or in moist chambers. Keeping the soil well watered by placing the pots of iris in pans of water is not sufficient.

The leaf blades were injured prior to inoculating them with the bacteria. A small instrument, an insect needle, was used, since anything as large as a sharp-pointed scalpel will cause an injury so great that a necrotic lesion may develop even in the absence of the bacteria.

The bacteria, for inoculation purposes, were grown on beef-extract-peptone agar and incubated at 27° C. for approximately 48 hours. They were then applied by different methods to the injured leaves. A water suspension of the bacteria was either sprayed on with an atomizer or applied directly with a camel-hair brush. The latter method is simple and effective. The bacteria also may be smeared on the leaf directly from the agar culture with any convenient implement.

In the majority of cases where the leaves are injured infection takes place. Under dry conditions infection is light, and in some instances the results are negative. Under moist conditions lesions may appear within a week, but the progress of the disease is slow. The so-called incubation period, however, appears to vary considerably. This is due, no doubt, to the fact that infection may take place with no visible lesion, that is, the pathogen may establish itself in the vascular system, but cause no necrosis nor discoloration of the tissue. We have here what one might call a latent infection or a symptomless infection. In one experiment, a pot of inoculated iris remained on the greenhouse bench for 2 months without showing visible symptoms. The pot was then placed in a moist chamber, and in a few days water-soaked lesions began to develop on the foliage.

Many inoculation experiments were conducted on the rhizomes of the iris with the pathogen, but no visible symptoms developed. Nevertheless, the following experiment was conducted to determine whether or not the rhizomes might harbor the pathogen without showing visible symptoms and later give rise to infected leaves. A series of 4 plants was used and the leaves were cut back to the rhizomes. These plants then were inoculated by piercing the rhizomes with a sharp pointed scalpel loaded with the pathogen. Recovery was immediate and new leaves were sent forth. These plants were held under conditions favorable for the development of the disease and 3½ months later were examined, both in the rhizomes and in the leaves. There was no apparent infection, and microscopic examinations yielded no bacteria. It is probable that the disease does not extend below the leaves.

Cross-inoculation experiments were made on a number of species of plants outside the genus *Iris*. The plants used were of 2 groups, i.e., species in closely related genera; and plants infected by related pathogens. The

technique used was that described above. Three attempts were made to infect the blackberry lily, *Belamcanda chinensis*, DC. a close relative of the iris, but the species appeared highly resistant, and in only one case was there any suspicion of infection. In this instance, the plants were exposed to a temperature of approximately 78° F. and humid conditions. A slight water-soaked area appeared about the infection court, but developed no further. Inoculations on the white trumpet lily, *Lilium longiflorum* Thunb., also were without results.

Since the pathogen in culture is so similar in appearance to *Phytomonas stewarti* (E.F.S.) Bergey *et al.*, *Phyt. michiganensis* (E.F.S.) Bergey *et al.*, and *Phyt. flaccumfaciens* (Hedges) Bergey *et al.*, attempts were made to infect the hosts of these 3 pathogens, sweet corn, *Zea mays* L. var. *rugosa* Bonaf.; the tomato, *Lycopersicon esculentum*, Mill.; and the bean, *Phaseolus vulgaris* L. Three different isolates of the iris organism were used and approximately 24 plants of each host species. Inoculations were made at 2 different dates. In no instance was there any sign of infection.

#### THE PATHOGEN

Approximately a dozen isolates have been in culture from time to time, but in describing the bacterium only 3 were used. Two of these were isolated from different specimens early in the summer of 1935, and the third was a reisolation of one of these. All 3 behaved the same.

*Morphology:* The pathogen is a small rod, occurring singly or in pairs. Cultures on beef-extract-peptone agar (pH 6.8–7.00), incubated at 27° C. for 36 hours, showed the following dimensions for the cells: 1.58  $\mu$  (1.05 to 2.45  $\mu$ ) by .68  $\mu$  (.47 to 1.05  $\mu$ ). A congo red negative stain was used in these measurements.

The organism is motile by a single polar flagellum that is twice or more the length of the cell. The pathogen is Gram-negative.

*Cultural Characteristics:* On beef-extract-peptone agar (pH 6.8–7.00) colonies are circular, entire, and 1 to 1.5 mm. in diameter. They are a mustard yellow. Isolates that have been grown in culture for some months produce on slants of the above medium a very moderate growth, which may appear in 24 hours. The streaks are filiform, glistening, and butyrous in consistency. In beef-extract-peptone bouillon (pH 6.8), clouding is fair, and in Clara's medium (3) the clouding is light. In brom creosol purple milk, growth is slow and at the end of a month the medium is bluish purple, but otherwise unchanged. Clearing becomes evident in 5 to 6 weeks. In shake cultures with a beef-extract-peptone agar plus .5 per cent dextrose used as a medium, colonies appeared on the surface or only 1 mm. below, showing that the pathogen is an aerobe.

*Biochemical Reactions:* A study of certain biochemical reactions of the pathogen in culture was made and the various reactions are as follows:

Growth in a gelatin stab culture is fair, but no liquefaction takes place. Growth is fair in the synthetic nitrate medium listed in the Manual of Methods (5, Leaflet II, Ed. 6, 1936, p. 15) when sodium citrate is substituted for the dextrose. Tests for nitrites with sulfonilic acid and  $\alpha$ -naphthalamine in acetic acid, give a positive reaction. The pathogen, when grown in beef-extract peptone broth, produces ammonia. Tests have been made after the Hansen method (5, Leaflet VI, Ed. 6, 1935, p. 12). To determine whether or not the bacteria produce hydrogen sulphide, they were grown in Bacto-triptophane broth, and strips of filter paper impregnated with lead acetate were suspended from the plugs, as recommended by Zobell and Feltham (5, Leaflet V, Ed. 5, 1934, p. 19). At the end of 3 weeks a very faint browning appeared on the lower edge of the filter paper. This test was repeated several times with no better success, although other organisms on the same batch of medium gave a strong hydrogen sulphide reaction. The pathogen, also grown in the same broth, was tested for indol production on the 1st, 2nd, and 7th days. The Ehrlich-Böhme test was used, and was negative in every instance.

Extensive carbon-utilization studies were made with the pathogen, but with somewhat unsatisfactory results. The bacteria grow poorly or not at all in certain synthetic media to which various carbon sources have been added. The pathogen does not grow in Ayers', Rupp's and Johnson's medium, (1) in the synthetic carbohydrate medium recommended in the Manual of Methods (5, Leaflet II, Ed. 6, 1936, p. 14), or in the nitrate synthetic medium of the same manual when dextrose is used as an energy source. Nevertheless, when certain other carbohydrates and certain sodium salts of organic acids are added to these synthetic media a light growth and a definite change in hydrogen-ion concentration takes place. It generally is considered that if an organism can utilize any carbohydrate it can utilize dextrose. Under the conditions of these experiments the pathogen, however, appears incapable of fermenting dextrose, but it does not necessarily follow that it can not ferment this sugar under other conditions not employed. With this in mind a further attempt was made to find a medium in which growth and dextrose fermentation might take place. It was known that a good growth occurs in beef-extract-peptone broth, although a definite alkaline reaction takes place in this medium due probably to ammonia production. This alkaline reaction would necessarily mask any acid from the fermentation of a carbohydrate in the strength that the peptone usually is used. However, it was thought that a very small amount of peptone might be added to one of the above synthetic media, sufficient to promote growth,

but not sufficient to produce enough ammonia to neutralize any acid that resulted from dextrose fermentation. Therefore, the synthetic carbohydrate medium mentioned above was employed and .05 per cent peptone added. Four lots of this medium were prepared and dextrose, glycerol, mannitol, and salicin were added separately in 1 per cent amounts. The 3 different isolates of the iris pathogen were transferred to 2 tubes each, of the various carbon sources. The pH of the media was approximately 7, with brom thymol blue added as an indicator. The cultures were incubated at 27° C., and after several days a light growth appeared in all tubes. Nevertheless, after a month's time there was no apparent change in the hydrogen-ion concentration, which indicated that these carbohydrates had not been used. It was unfortunate that the above carbon sources were used in this experiment, since it was later learned that certain other carbohydrates could be fermented in synthetic media, but not the 4 tested above.

A number of carbon sources were tested in various synthetic media. These were first, Ayers, Rupp and Johnson (1); second, the synthetic carbohydrate medium of the Manual of Methods (5, Leaflet II, Ed. 6, 1936, p. 14); and third, the synthetic nitrate medium listed in this same Manual (5, Leaflet II, Ed. 6, 1936, p. 15). As a rule, the media were sterilized with steam at 15 pounds' pressure for 20 minutes. When levulose, arabinose, xylose, lactose, maltose, or sucrose was added, sterilization was affected through filtration, with a Berkefeld filter (N). Brom thymol blue was used as an indicator and the pH varied with the lot of media from 6.8 to 7.0. All tubes were incubated at 27° C.

Following are the results of the tests. Each carbon source was not always used in all 3 media, and is reported only as used. The numbers refer to the media as listed above. Dextrose, no growth in 1, 2, or 3; levulose, growth and acid production in 2 and 3; galactose, no growth in 2, good growth and acid production in 3; arabinose, no growth in 2, growth and acid production in 3; xylose, doubtful growth in 2, growth in 3; rhamnose, growth in 3; lactose, no growth in 2 or in 3; maltose, growth and alkali production in 2 and in 3; sucrose, no growth in 2 or in 3; mannitol, no growth in 2, but doubtful in 3; glycerol, no growth in 1 or 3; salicin, no growth in 3; sodium acetate, no growth in 1; sodium citrate, growth and alkali production in 1, 2, and 3; sodium formate, no growth in 2; sodium lactate, no growth in 1; sodium malate, growth and alkali production in 1; sodium succinate, growth and alkali production in 1; sodium tartrate, no growth in 1. The pathogen grew well on the standard beef-extract-peptone starch agar, but starch was not hydrolyzed.

#### TAXONOMY

A number of bacterial species have been reported in literature as pathogenic to the iris. Miss Elliott (4), in her Manual, lists the following:

*Erwinia caratovora*, *E. aroedeae*, *Phytomonas iridis*, *Phyt. gladioli*, and *Phyt. marginata*. The first 3 species cause a soft rot of the rhizomes and of the lower leaf sheaths and can not be confused with the pathogen and disease under consideration. *Phyt. marginata* and *Phyt. gladioli* occur on the leaves, but the latter has been observed only from artificial inoculations. Neither of the 2 pathogens resemble the iris blight organism.

Takimoto (6) in 1932 reported a leaf spot of *Iris tectorum* and *I. japonica* due to a bacterium that he described and named *Phytomonas iridicola*. This pathogen forms white colonies, liquefies gelatin, and hydrolyzes starch, which in no way agrees with the behavior of the blight pathogen.

As stated previously, the iris pathogen resembles in culture *Phytomonas stewarti*, *Phyt. flaccumfaciens* and *Phyt. michiganensis*. Cross inoculations were all negative, however, which proves a pathogenic difference. Differences in motility and the reaction to the Gram stain are also in evidence.

Since this article was accepted for publication, Miss L. McCulloch presented a paper on a bacterial leaf disease of iris at the Christmas meetings (1936) of the American Phytopathological Society. The abstracts of her paper leaves little doubt that we have been working with the same disease. Miss McCulloch named the pathogen, *Bacterium tardicrescens*, and since this name will be in print before the present article is published, I have made certain changes herein to comply with it, therefore avoiding a synonym. I am using, however, the Bergey classification and propose the name as follows, *Phytomonas tardicrescens* (McCulloch) n. comb.

This species is placed in the genus *Phytomonas*, but it already has been pointed out by the writer (2) that this genus is composed of a very heterogeneous group of bacteria. The relationships of the iris pathogen within this genus are with the *Stewarti* group, which is composed mainly of vascular parasites. These pathogens are *Phyt. stewarti*, *Phyt. flaccumfaciens*, *Phyt. insidiosa*, *Phyt. michiganensis*, *Phyt. rathayi*, and possibly several others not fully described. These relationships are pointed out here with the hope that in the future taxonomists will recognize the grouping.

#### BRIEF DESCRIPTION

*Phytomonas tardicrescens* is a non-spore-forming rod, occurring singly or in pairs. Single cells average  $1.58\ \mu$  ( $1.05$  to  $2.45\ \mu$ ) by  $.68\ \mu$  ( $.47$  to  $1.05\ \mu$ ). It is motile by a single polar flagellum, is Gram-negative and aerobic. On beef-extract-peptone agar surface colonies are circular, entire, and 1.0 to 1.5 mm. in diameter. Their color is a mustard yellow. In bromocresol purple milk, growth is slow, the milk turns alkaline, and eventually clears. Gelatin is not liquefied. Nitrates are reduced to nitrites. Ammonia is produced and a trace of hydrogen sulphide. No indol is produced.



Levulose, galactose, arabinose, xylose, rhamnose, and the sodium salts of citric, malic, and succinic acid are fermented. Starch is not hydrolyzed.

The organism is pathogenic on *Iris germanica*, *I. florentina*, and *I. fulva*.

#### SUMMARY

A bacterial blight of iris possibly of widespread distribution and one that may be confused with other diseases of this plant is described. The disease is favored by wet weather and occurs on at least 3 varieties of iris. The causal organism, a vascular parasite, was isolated and its pathogenicity proved. A description is given of the organism in culture and the name *Phytomonas tardicrescens* (McCulloch) n. comb., is proposed.

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## PROGRESS IN PLANT PATHOLOGY: CONTROL OF DISEASE BY RESISTANT VARIETIES<sup>1</sup>

G. H. COONS

That definite progress in plant pathology and an advance toward the ultimate control of plant disease have been shown by the development of resistant varieties certainly will have the assent of this audience. In the case of one plant or another, we are cognizant of the extent of this progress. But I am not sure that the magnitude of the contribution to human welfare that has come about is recognized. It will be one purpose of this discussion to explore the situation as it exists today. Then it will be appropriate to consider some of the salient principles that have come out of the research and finally to comment on some responsibilities that rest upon us as phytopathologists.

The 1936 Yearbook of the U. S. Department of Agriculture is devoted to an inventory of superior germ plasm of crop plants and farm animals. In this presentation, one is struck by the emphasis given by the plant workers to disease resistance. There also has come to you from Haskell, Boswell, Doolittle, Porte, Harter, Emsweller, and Weiss "Sources of Disease-Resistant Vegetable and Flower Seeds, 1936".<sup>2</sup> This inventory, which deals in the main with varieties catalogued by seedsmen, and thus available for the grower's use, is impressive with respect to the range of cultivated species covered and the numbers of resistant varieties within a species offered.

Truly, the expansion of this branch of plant-disease control since the pioneer work of Orton and Bolley, carried forward with the impetus and inspiration given by the work of Bain, Essary, Freeman, Johnson, Jones, Norton and others, demands our admiration. But I shall not yield to the temptation to make this address a historical resumé, fascinating as it might be to start with speculation on what made Achilles barley, mentioned by Theophrastus, more susceptible to rust than its contemporary varieties. Let others conjecture the physiological race that, in those bygone days, was responsible for the discomfiture of the quondam sponsor of this variety—the "Conqueror" or "Hero" of its day. Actually, this whole matter of disease resistance as a control method for plant disease is almost too young to have history. It has taken place within the memory of many of us. The classic researches of Orton on cotton, cowpea, and watermelon wilt should need no review. These should be required reading for every phytopathologist.

<sup>1</sup> Address of the retiring President of The American Phytopathological Society, delivered Dec. 30, 1936.

<sup>2</sup> Extension Service and Bureau of Plant Industry, [U.S.D.A.] publication, 41 pp. (mimeographed).

In the consideration of this subject, I shall seek some measure of the returns that have come about from the use of disease resistant varieties. In confining this to the present period, some enormously important examples, far-reaching in their influence on the development of this plant-disease-control measure, are passed over. These, however, are the classic illustrations of your lectures. In attempting to make a factual approach, such data or estimates as have come readily to hand have been marshalled to furnish an evaluation of the contribution assignable to disease resistance. Where authoritative estimate is lacking, you will find I have had the temerity to supply it. The standard crop statistics for acreages, yields, and farm values for certain important crops, taken as an 8-year average, served as the base of these estimates. From the 1936 Yearbook, one may glean estimates of acreages devoted to one variety or another for many of the important field crops. Some of these are characterized as disease-resistant; and critical studies on many of these varieties point out their respective virtues and failings. For other crops, from one bit of evidence or another, an estimate is made of the proportion of the acreage planted to resistant varieties. These have been checked, in many instances, by persons familiar with the crop for plausibility of the estimates.<sup>3</sup>

The totals secured are of interest. The average annual acreage of 17 important crops, for the period 1928 to 1935, was about 211,400,000 acres. The acreage for disease-resistant crops, estimated as I have indicated, reached in 1935, 55,555,000 acres. Obviously, this acreage varies as varieties come into prominence and shifts from year to year, but it seems always on the gain. In other words, at present on nearly 1/4 of the total farm acreage, varieties recognized as disease-resistant are in use. Of a total farm value of crops estimated at about \$2,500,000,000, disease-resistant varieties would, at the same rate per acre, account for \$618,000,000 of estimated farm value. It is to this last-named figure that we wish to turn attention.

What was the money benefit that those 55,555,000 acres of recognizably resistant sorts gave by virtue of their disease-resistance quality, over what would have come from the varieties that were displaced? Obviously, there is no way to get at this but by a sort of guess. If one turns to comparative variety tests, gains are recorded that do not materialize in large-scale use, often because the disease effects are not severe or widespread enough to bring into play the full force of the improved variety. If one were to hazard an "estimate", where may the average benefit be placed—at 1 per cent, 5 per cent, 10 per cent, or more? I should like to make the assumption that the contribution to farm values from the use of recognizably disease-resistant

<sup>3</sup> Acknowledgment is made to S. C. Salmon, J. A. Clark, T. R. Stanton, M. T. Jenkins, V. R. Boswell, H. A. Jones, and R. J. Haskell of the U. S. Department of Agriculture for assistance in this connection.

sorts should, on the average value of the 17 crops taken as a whole, amount to at least 10 per cent. For some crops, this figure may be high, but for other crops it is obviously exceedingly low, since there are numerous crops where disease resistance makes the difference between success and failure. My predilection for the 10 per cent figure comes, I presume, from a belief that a disease-resistant variety in station or grower tests must be superior by at least such an amount for this superiority to be recognized.

A more analytical method of approach would seem preferable. Here, instead of a general estimate, the crops may be considered individually. From a general judgment on varietal behavior and disease loss, and with a conservative placing of the contribution that the existing degree of resistance may be expected to make, one can, for each crop, estimate a specific contribution, hoping this will give a better-founded figure.

In table 1, I present such estimates. I shall have accomplished my purpose if the figure for percentage benefit appears to you, for the crops with which you are familiar, as entirely too low.

Allow me to illustrate the method used in deriving these figures by a few examples. The corn crop is majestic in its acreage and without equal in its contribution to national wealth. Disease losses for this crop arise chiefly from corn smut and the stalk and ear rots. I have assumed that only in the acreages planted with  $F_1$  hybrids should we expect benefit to come from disease resistance. In the 1936 Yearbook, approximately 50 per cent of the inbred lines are indicated as exceeding the standard in smut resistance or in resistance to stalk or ear rots when used in hybrids. Hybrids of station lines were used on approximately 195,000 commercial acres in 1935. Of course, a much larger acreage was planted with  $F_1$  hybrids from commercial breeding establishments but their resistance qualities are not recorded. I have assumed that half of the hybrids from station lines would show some measure of resistance to corn diseases, and have placed the benefit at merely 2 per cent. The 1935 contribution may seem a small one, but, with the rapid increase in acreage in which  $F_1$  hybrid corn is planted, a future tabulation will tell a far different story.

The whole history of wheat in the great wheat regions has shown the steady march of improved varieties and in this improvement, disease resistance has been especially stressed. As I consult the 1936 Yearbook, I find numerous varieties, the wheel horses in wheat growing, characterized as having some measure of resistance to rust, bunt, or loose smut. Thus, in 1934, the Turkey wheats are estimated to occupy 14,828,000 acres; Marquis, 8,510,000; Ceres, 4,453,000; Kanred, 2,298,000; Trumbull, 1,135,000; Kubanka, 667,000; Albit, 392,000. The varieties Tenmarq, Marquillo, Minturki, Forward, Berkeley Rock, Acme, Hope, Denton, Progress and Thatcher together occupied about 1,000,000 acres. Each of these varieties has its measure of resistance to some

TABLE 1.—*Disease-resistant varieties and their contribution*

Crop	Acreage (1928-35 Ave.) (approx.)	Farm value (1928-1935 Ave.)		1935 acreage in disease-resistant varieties (estimated)	Average farm value of resis- tant crop (estimated)	Estimated benefit from disease resistance	
		Total (approx.)	Acre (approx.)			%	\$
Corn .....	99,702,000	\$1,364,900,000	\$ 14.00	100,000	\$ 1,400,000	2	\$ 28,000
Wheat .....	54,926,000	538,000,000	9.80	39,000,000	382,200,000	5	19,110,000
Oats .....	38,254,000	334,700,000	8.75	10,000,000	87,500,000	5	4,375,000
Barley .....	11,707,000	105,300,000	9.00	2,500,000	22,500,000	2	450,000
Flaxseed .....	2,269,000	22,269,000	10.00	2,000,000	20,000,000	25	5,000,000
Beans (dry) .....	1,716,000	42,800,000	25.00	900,000	22,500,000	5	1,125,000
Sugar cane (La.) .....	253,000 <sup>a</sup>	12,100,000 <sup>a</sup>	47.80 <sup>a</sup>	250,000	11,950,000	50	5,975,000
Sugar beets .....	763,000	49,600,000	65.00	102,000	6,630,000	25	1,657,000
Asparagus .....	103,400	12,400,000	120.00	95,000	11,400,000	50	5,700,000
Cabbage .....	144,900	14,000,000	97.00	14,000	1,358,000	50	679,000
Cantaloups .....	116,600	15,700,000	135.00	38,000	5,130,000	Total	5,130,000
Celery .....	32,600	13,700,000	420.00	1,000	420,000	50	210,000
Corn (sweet) .....	327,000	6,500,000	20.00	100,000	2,000,000	5	100,000
Lettuce .....	153,000	28,400,000	185.00	90,000	16,650,000	75	12,487,000
Peas .....	327,600	18,700,000	57.00	150,000	8,550,000	10	855,000
Spinach .....	63,900	6,000,000	95.00	15,000	1,425,000	50	712,000
Tomatoes .....	490,000	40,000,000	82.00	200,000	16,400,000	15	2,460,000
Total .....	211,349,000	\$2,625,169,000	.....	55,555,000	\$618,013,000	.....	\$66,053,000

<sup>a</sup> Estimates for 1934 and 1935.

of the important pathogens; I have believed it conservative to say that, by and large, these varieties must have made the crop at least 5 per cent greater by virtue of their disease resistance than if the varieties they displaced were grown.

And so we go through the list and consider each item. In certain cases, we know the disease-resistant variety means the difference between success and failure. Here I have placed the contribution high—but not so high as actual comparison of resistant and general run would warrant. Again, I suggest the conservatism of my figures.

If you have concurred, or not too violently dissented so far, you are ready for the summing, and we arrive at a figure of \$66,000,000. As an estimate, therefore, of the contributions of varieties as chosen today for their resistance, grown on this enormous acreage, aggregating nearly  $1/4$  of the acreage devoted to the crops considered, I do not hesitate to place the annual contribution to farm wealth as not less than \$60,000,000 to \$70,000,000. If you think the contribution should be placed at a higher figure, I would be content to accept this also.

But, there may be those who are skeptic and ready to question the estimates of total acreage, the estimates of acreages in resistant varieties, the loss estimates, and the estimates as to contribution. Frankly, data, surveys, and close field estimates do not exist with which to prove the case.

But, for two crops, we have specific information allowing us to defend the position that use of resistant varieties can give positive, decisive, and almost revolutionary effects. I refer to sugar cane and sugar beets. In these cases, we are dealing first with crop statistics that are exceptional in that these crops, grown under contract, are reported as actual acreages grown. The fields are measured, the returns of each grower are weighed, and the data compiled by the Department of Agriculture. We know positively that hardly an acre of nonresistant sugar cane is grown. In the case of sugar beets, we have the figures compiled by the industry, indicating accurately the actual acreage, and furthermore, we know, almost to the pound, the quantity of seed of the resistant variety available.

I am indebted to R. D. Rands for a statement of the actual situation with respect to the influence of resistant varieties of sugarcane. "The general falling off of yields and repeated crop failures during the period 1923 to 1927, in Louisiana, resulted in virtual bankruptcy of the historic sugar industry in that state. This was due to the combined effect of three major sugarcane diseases: mosaic, red rot, and root rot, to all of which the old 'noble' varieties of cane were very susceptible. . . . The decline in both acreage and production from the high level between 1904 and 1911 to the disastrous low point in 1926 and 1927 is shown in figure 1.

"Introduction of improved varieties, beginning in 1924, resulted by 1928 in almost complete change in the varieties grown. From the disastrous average yield of 6.8 tons of cane per acre in 1926, which was the last year the Louisiana crop was made exclusively from old varieties, yields climbed to 16.2 tons in 1928 and 18.8 tons in 1929. The 1928 crop was estimated as worth about \$21,000,000 as compared with \$7,000,000 from a materially larger acreage of the old varieties in 1926."

The situation is shown by average acre yields of sugar for Louisiana in which the 7-year period, 1922-1928, is contrasted with the 7-year period, 1929-1935. The average production of sugar per acre in the period when susceptible varieties were used, and when the transition from susceptible to improved varieties was taking place, was 1,636 pounds; the average for the

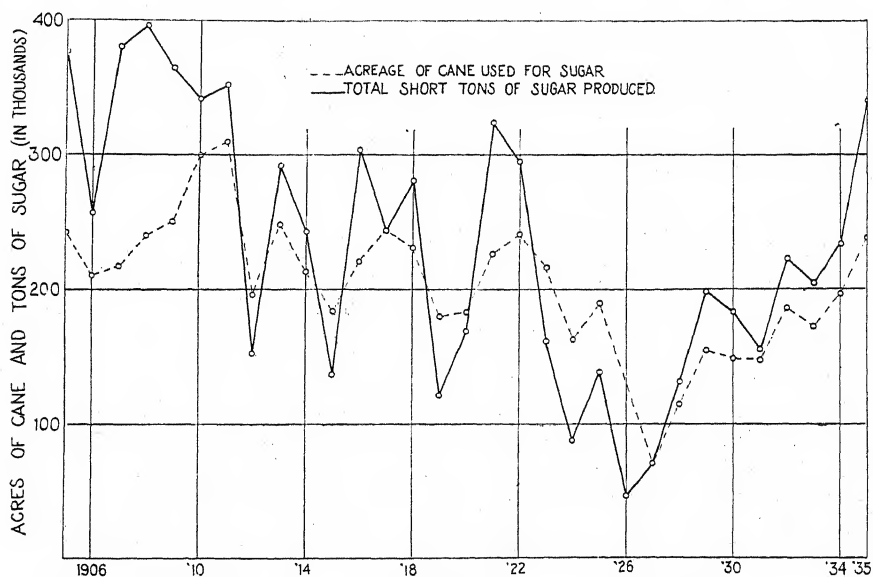


FIG. 1. Acreages of sugar cane in Louisiana and tonnages produced for period 1905-1935, inclusive. (Prepared by R. D. Rands.)

7-year period 1929-1935, in which the use of P.O.J. varieties (tolerant and partially resistant) and the newer, more resistant canes became general was 2,440, a gain of 804 pounds per acre, or nearly 50 per cent. For this crop, at least, the estimated year-by-year gain from disease resistance attributable to the new varieties, when placed at 50 per cent is demonstrable as conservative.

In the case of sugar beets, I can refer to the results from introduction of the curly-top-resistant varieties, U. S. 1, U. S. 33, and U. S. 34. We know from factory records that, in 1934, approximately 35,000 acres were planted with U. S. 1; in 1935, the acreage was 102,803 acres; and in 1936, at least as

much. In 1937, upwards of 150,000 acres will be planted with U. S. 33, U. S. 34, and A.S.C. 600, all curly-top-resistant varieties.

Tests with U. S. 1 have shown that an average superiority of 5 tons per acre may be expected under conditions of moderate to severe exposure over results from European brands. Tests in many States with a variety of exposures indicate an average contribution for U. S. 1 under conditions of curly top as may exist—light, moderate to severe—is safely placed at 2.5 to 3 tons, approximately 25 per cent of an average crop. With the newer varieties, the contribution is even greater. For 1935, a year of severe curly top in many areas, the resistant varieties produced a harvestable crop with some damage by curly top, the European brands were a practical failure.

I would call attention to the many crops that, for lack of data, have not entered into this calculation: potatoes, cotton, tobacco, alfalfa and other forage crops, and the numerous minor vegetables and flowers. Haskell *et al.* lists, in addition to the major vegetable crops included in the above tabulation, such crops as snap and red kidney beans, resistant to rust, mosaic, blight, halo blight; pumpkin, squash, resistant to curly top; watermelons, resistant to wilt; and for the flowers, wilt-resistant asters, and rust-resistant snap dragons.

In arriving at the figure for contribution from disease resistance, I have considered this by itself. This is a segregation of benefits that perhaps is not justified except for such purpose as this. The contribution of a resistant variety includes many other things: Improvement, both in yield and quality, usually has accompanied the resistant variety, irrespective of resistance; certainly, those that have gained ready acceptance, such as Washington asparagus, the Wisconsin Hollander cabbage, the various wheat varieties, Michigan Golden celery, and the newer sugar canes, have had improved commercial quality and high capacity for yield. Then, with the resistant varieties, even if the crop is somewhat reduced, there is at least something to harvest—something to offset labor costs in production, a striking contrast to crops not worth harvesting at all, as often occurred with the old varieties. Those intangible things, stability in rotational systems, permanence in the agricultural program of an area, and the measure of security that removes something of the hazard from crop growing and allows the farmer to plan with some confidence, are uncounted assets to the credit of disease resistance.

Furthermore, in basing the benefit on farm value of the crop, one whole side of the picture as it applies to national wealth is omitted. In a sugar-beet area, if farmers lose their crop from curly top, the beet factory loses by not being able to make sugar. With the majority of crops, it is my opinion that we would not be far wrong in placing the contribution to national wealth at double that computed in terms of the farm value.

Are we then drawing the long bow when we maintain that at present, with the plant-disease situation as it varies from year to year, the disease-



resistant varieties,—in their present status, which we all recognize is but a step to what is expected of accomplishment—are adding to farm wealth at least \$60,000,000 to \$70,000,000 a year and to national wealth a far greater figure?

If you have gone with me thus far, that the stakes we are playing for are high and that this is a matter of paramount importance in plant-disease control, then I am ready, in the interests of plant improvement, to approach the subject as to varieties now extant and be as critical as anyone. I would only call attention to these stupendous gains at this early state of this effort, obtained with the far-from-perfected varieties as they now exist, as an invitation to renewed and concentrated attack on the problems.

It is appropriate, in a consideration of progress in this field of plant-disease control, to comment upon some of the principles that have been established. Foremost of these, in my opinion, is the conclusive demonstration that within the host-plant complex we may confidently expect to find biotypes that differ from their neighbors with respect to resistance. It would seem to me that such a quality as disease resistance must have been an important element in the evolutionary process, determinative and positive. Hence it would not appear unwarranted, in view of the demonstrated cases of the existence of factors for disease resistance extending over the whole range of higher plants, to rely upon a rather general existence of such genes, or combinations of genes, within the species or genus and to weave this concept into our philosophy of disease control.

As a corollary to such a proposition, we may expect that, as we study the host complex, resistance phenomena will manifest themselves by forms showing the whole range of reactions extending from susceptibility to complete immunity. This, I believe, is in accord with experience in such self-pollinated plants as wheat, beans, and those propagated vegetatively—a collection of varieties falling into the normal distribution curves with respect to the disease reactions shown toward a given pathogen. With the cross-pollinated plants, because of the commonly heterozygous condition within the recognized varieties, we need to push our analysis to the reactions to the individual plants; but, here again, these individuals arrange themselves in such a distribution curve. With curly top, anyone viewing a field of sugar beets where the disease is severe would, perhaps, have felt that here optimism that resistant individuals could be found was unjustified. Yet, even in the worst fields, individuals completely exposed to infection survived, and the field records of individual plant reactions show this distribution, askew, to be sure, toward susceptibility, but still with resistant forms represented in the population.

The breeding work with sugar beets of the late W. W. Tracy, Jr., had concerned itself over many years with separating from the sugar-beet complex some 750 selected or inbred lines more or less tending to homozygosity with respect to certain factors. When a part of this collection, selected entirely

on agronomic or morphological characters, was exposed to curly-top epidemic some lines proved extremely susceptible, the majority were susceptible, but a dozen or more lines showed conspicuous resistance. Of course, this represents merely the same phenomenon previously encountered with individuals. The plant breeder had, by his isolation technic, established in partly purified state the biotypes extant within the sugar-beet population. These showed the variation in disease response previously manifested by the individuals in a population.

Much research has now been done on material obtained in just this way, and in the resistant lines derived from the primary selections. Evidence is piling up, showing that resistance can be assigned to genetic factors. As study proceeds, more and more cases are found to show that the factors for resistance form an allelomorphic series, but no uniformity exists among the plant species and obviously none is to be expected. As experience accumulates, the necessity of combining the factors for resistance with those for quality and yield becomes clear. It seems safe to predict that, if work is done with necessary numbers and with varieties best suited to make desirable contributions, this combining of qualities can be accomplished. As biotypes are isolated in certain plants and pure lines established, which are stabilized for resistance, loss of vigor may be encountered. Here hybridization between satisfactorily resistant lines must be made in order to permit utilization of hybrid vigor. In short, every advance in plant breeding and genetics needs to be called into use in meeting this ever-expanding problem.

We are concerned with the phytopathological phases. It is apparent from what has been said that the individuals set apart from their neighbors by virtue of their resistant reactions are the building stones in this structure. If such bearers of the factors for resistance can be found within the cultivated forms as they exist, the job is much simplified. Otherwise the forms to contribute resistance must be sought in the related species of the genus, including the wild progenitors. The study and determination of the relative resistance of the host plant, considered in its broadest sense, and its botanical allies are fundamental in the program, and such search and study should engage the phytopathologist.

Discovery of these relationships is not a simple matter, nor one that can be solved by casual observation. The work of the last two decades has given abundant evidence that complexity of the host is paralleled by complexity in the pathogens. The researches on physiological races, on variants, on new combinations, and on mutants within the pathogenic species or within the viruses are accumulating information that must be taken into account because of direct bearing on future progress in disease resistance. To some, the enormous potentialities for variability in the pathogens has led to a rather pessimistic viewpoint toward the whole matter of disease resistance. It is obvious that this attitude is a reaction against a doctrine that the whole

matter of disease resistance is merely a matter of juggling a few genes, and—*presto*, there is the answer! The measure of success already obtained, sustained as it is over many years, carries elements to contradict a too pessimistic view. But we must expect that a succession of varieties, and a localization in some cases of the adaptability of a variety, will result from the capacity of this other biological entity (the parasite) in the disease complex to vary.

The methods successful in the past have, in part, taken into account the complexity of the pathogen and sought to have the exposure involve as large a section of the pathogen population as possible. Fair inference from the recent research clearly justifies still more insistence that breeding work be conducted so as to deal with the broadest possible range of biotypes of the parasite. Neglect of this, which can easily come about by diminution of the interest of phytopathologists in this field of plant-disease control, is going to result in varieties that represent scientific stunts rather than distinct contributions to our agriculture.

In passing, I want to comment on another phase of phytopathological contribution to this matter of disease-resistance breeding, namely, the matter of disease exposure. As one reads the older reports, he is struck by the fact that the successful isolations of resistant individuals were made in fields where the disease exposure approached 100 per cent. Such was the case with cotton wilt, flax wilt, and with cabbage yellows. Orton induced such conditions in his work with watermelon wilt. The phytopathologist has a necessary place in a disease-resistance program, in producing such conditions of exposure, that by intensity of infection and by range of pathogenic forms involved, the lucky plants that escape infection are not taken to hamper and impede the work. There is a distinct need for contributions by phytopathologists to the matter of inoculation methods, especially field inoculation. We need, from our study of epidemics and by experiment, to learn how to produce epidemic conditions.

And, as a closing portion of these remarks, I want to touch on another phase, the nature of plant-disease resistance. At present, we are dealing with host plants that we know imperfectly, exposing them to pathogens that we also know very imperfectly, expecting to move about genes, which we postulate and that produce, in some unknown way, effects on some unknown characteristic of protoplasm that imparts disease resistance. As these problems are elucidated, it behooves us to make our contribution as to the nature of plant-disease resistance.

The recent classification of resistance into mechanical, physiological, and functional represents a worthwhile setting out of the categories. I might express a wish that the lessened disease resulting from what I call "commercial resistance", which isn't resistance at all but escape, due to earliness, vigor, non-bruisibility, etc., might be appropriately named, set off by itself, to reduce confusion of thought.

There is considerable literature on the nature of disease resistance. With some plants, resistance has been assigned to tolerance to toxic substances produced by fungi or bacteria; with others, it may be correlated with the reaction of the plant juices. Possession of certain chemical constituents has been shown to be involved in the case of other plants. The inability of the pathogen to appropriate nitrogen from the plant proteins by its proteolytic enzymes may possibly be a factor in some cases. There are undoubtedly a number of resistance phenomena connected with the matter of invasion and subsequent advance into the tissues, including the classic case of hypersensitivity conferring resistance. The recent review by Brown<sup>3</sup> shows the size of this field of research, the wide range of factors concerned, and, in general, the need for more intensive research. All in all, many factors are reported as bases for resistance; as research proceeds, one can expect this complexity to be even more fully shown. In any program of disease-resistance breeding the phytopathologist must contribute discoveries applicable to the problem at hand. For any given pathogen, fuller knowledge as to the factors underlying manifestation of disease resistance may have guiding influence on the methods to be employed in securing resistant plants. Phytopathologists have a distinct responsibility to press actively forward toward the solution of this fundamental problem of our science.

I have sought to evaluate the progress made in securing resistant varieties of our important crop plants. I believe I have shown by conservative estimate that already enormous contribution to national wealth is being made, even with the measure of disease resistance secured. It forecasts the possibilities as research goes forward. I have outlined the scientific findings that have come from the work done. That resistant biotypes may be expected to exist within the complex plant material seems clear; these await discovery and utilization. But the job is not so simple as it sounds, because of the complexities found within the pathogens themselves; the varied potentialities of physiological races within the parasitic species, the new combinations, mutations, and the types of viruses, must be taken into account if the research is adequately to meet the complex problems of plant-disease control. In the fundamental phases of the program involving the pathogen, in the search and evaluation of foundation materials, and in the setting up of conditions of exposure, the phytopathologist has distinct contributions to make. In the determination of the particular factors underlying the resistance shown by a host plant, there is opportunity to make decisive and guiding contribution. In my opinion, it would be unfortunate for agriculture if phytopathological interest in this field should slacken.

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<sup>3</sup> Brown, W. The physiology of host-parasite relations. Bot. Rev. 2: 236-281. 1936.

# INFLUENCE OF SOIL TEMPERATURE AND SOIL MOISTURE ON INFECTION OF STEM SMUT OF RYE<sup>1</sup>

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(Accepted for publication Jan. 26, 1937)

Previous investigations have shown that soil conditions play an important rôle in infection and subsequent development of cereal smuts. Stem smut of rye, caused by *Urocystis occulta* (Wallr.) Rab., has received less attention than some of the other cereal smuts; therefore, experiments were made to determine the influence of soil temperature and soil moisture on infection by this smut. Preliminary experiments on the influence of temperature have been reported by Stakman, Moore, and Cassell (6).

## MATERIAL AND METHODS

Experiments were made in constant-temperature tanks, previously described (2). The experiments at 5° C. were made in a cold chamber because this temperature could not be maintained in the tanks.

The smut used was collected at University Farm, St. Paul, Minnesota, the year in which the experiments were made. The seed was inoculated by thoroughly dusting with chlamydospores and was planted in 6-inch clay pots or glazed half-gallon crocks, the latter being used when it was desired to control soil moisture. Special attention was paid to uniformity in compactness of soil and depth of planting. Seeds were not planted until the soil temperature became comparatively constant. After the seedlings had developed to the 2-leaf stage, all pots or crocks were removed from the tanks and kept under the same conditions in the greenhouse.

In 1932 Dakold rye was used. Plantings were made in 6-inch clay pots, and 6 pots were maintained at each of the temperatures, 14°, 19°, 24°, and 29° C. In 1934 the variety Rosen was used, and 4 glazed crocks were held at each of six temperatures ranging from 5° to 30° C. The soil was a mixture of 2 parts black loam and 1 part sand, and the moisture content was adjusted to approximately 25 per cent of the water-holding capacity in the first experiment and to approximately 25 per cent and 65 per cent in different parts of the second experiment. Moisture determinations were made on an air-dry basis. The seed was planted 1-inch deep in the first experiment and  $\frac{1}{2}$  inch deep in the second.

## EXPERIMENTAL RESULTS

Although the results obtained in 1932 and in 1934 vary somewhat, probably because different varieties of rye were used and greenhouse conditions differed, they indicate that infection is favored by relatively low soil tempera-

<sup>1</sup> Paper No. 1476 of the Scientific Journal Series, Minnesota Agricultural Experiment Station.

ture and soil moisture. The thermal range for infection was between 5° and 25° C., with optima varying from 13.5° to 17° C.

TABLE 1.—*The influence of soil temperature on infection of Dakold rye by Urocystis occulta in 1932<sup>a</sup>*

Temperature in degrees C.		Days required for emergence of seedlings	Smut after 81 days		Smut after 180 days	
Average	Range		Total plants	Per cent infected	Total plants	Per cent infected
14	11 -17	7	172	33	93 <sup>b</sup>	47
19	17.5-21	5	176	33	85	35
24	20 -26	4	204	17	99	24
29	27 -30.5	4	175	trace	94	0

<sup>a</sup> Moisture content of soil not determined.

<sup>b</sup> Plants from 3 of the 6 pots on which notes were taken after 81 days.

In 1932 the first smut count was made 81 days after planting. All the plants were pulled from 3 of the 6 pots at each temperature and examined for smut-striped leaves. From the remaining 3 pots at each temperature only the smutted plants were pulled and the apparently healthy plants were kept 99 days longer until they headed, to determine whether more smut would develop. From table 1 it appears that the optimum temperature for infection was 14° and the maximum 29° C. It appears also that smut subsequently developed in some plants seemingly healthy 81 days after planting.

In 1934, 2 experiments were made. Smutted plants in both cases were counted 3 months after planting, and again a month later, although, at the relatively high temperature of the greenhouse during these experiments, there was no further development of smut after the first 3 months. The results are summarized in table 2. In these experiments the optimum temperatures for infection were 17° C. when the seed was planted 1 inch deep in soil moistened to 25 per cent of its water-holding capacity, and 13° and 13.5° C., respectively, when the seed was planted  $\frac{1}{4}$  inch deep in soil moistened to 25 and 65 per cent of its water-holding capacity.

Under the various combinations of conditions in the different experiments in 1932 and 1934, 4 temperature optima were observed: 13°, 13.5°, 14°, and 17° C. In view of the unavoidable fluctuations in soil temperatures, the small differences between the first 3 probably are not significant. The higher optimum, 17° C., occurred when seed was planted 1 inch deep and can reasonably be attributed to depth of planting since it was the greatest apparent variable. Hecke (3) considers temperature as a factor affecting the duration of the susceptible stage of the host. Bartholomew and Jones (1) state that smut infection in oats may be correlated with the length of time required for seed germination. The optimum temperature for spore germination in *Urocystis occulta* has been found to vary from 15° to 18° C.<sup>2</sup> (5), and when the host remains in a susceptible condition long

<sup>2</sup> Ling, L. Factors affecting the development of *Urocystis occulta* (Wallr.) Rab. 1936. [Unpublished master's thesis, Univ. of Minnesota.]

TABLE 2.—*The influence of soil temperature and soil moisture on infection of Rosen rye by Urocystis occulta in 1934*

Depth of planting, 1 inch			Depth of planting, 4 inch		
Average temperature in degrees C. <sup>a</sup>	25 per cent soil moisture <sup>b</sup>		Average temperature in degrees C. <sup>a</sup>	25 per cent soil moisture <sup>b</sup>	
	Total plants	Per cent infected		Total plants	Per cent infected
5	55	4	5	41	5
13	73	22	13.5	43	30
17	74	39	18	46	21
21	83	11	20	52	19
25	73	7	26	43	0
31	61	0	30	41	0
				Total plants	Per cent infected
				13	0
				25	8
				21	5
				27	0
				15	0
				21	0

<sup>a</sup> The temperature prior to emergence of the seedlings did not deviate more than 2° C. from the average in any case.<sup>b</sup> Percentage of water-holding capacity of soil, determined on air-dry basis.

enough *i.e.*, the stage prior to emergence, this temperature could also be expected to be optimum for infection, as was actually the case when seed was planted 1 inch deep. However, when seed was planted only  $\frac{1}{4}$  inch deep, the time required for emergence, hence, the duration of susceptibility, was shortened and the optimum temperature might be expected to drop to a point low enough to lengthen the preemergence period.

High soil moisture, 65 per cent of the water-holding capacity of the soil, was unfavorable to seed germination, and there were fewer plants in this part of the experiment. Results, however, indicate that this degree of soil moisture distinctly hinders infection and also may limit the temperature range over which infection is possible.

Field observations agree well with results given above. Heavy infection of rye smut usually appears to be associated with relatively low soil temperature and fairly dry soil at time of planting. This is comparable with the observations of McAlpine (4) on flag smut of wheat, caused by *Urocystis tritici*.

#### SUMMARY

Repeated experiments were made in 1932 and 1934 in constant temperature tanks in the greenhouse to determine the influence of soil temperature and soil moisture on the infection of rye by *Urocystis occulta*.

Infection occurred commonly at soil temperatures from 5° to 25° C., with decidedly less infection toward the two extremes of the range.

Temperature optima for infection occurred from 13° to 17° C. and it is possible that the lower optima are correlated with shallow planting, the lower temperature prolonging the stage of susceptibility of the rye seedlings.

High soil moisture (65 per cent of water-holding capacity) reduced the amount of infection and also may have narrowed the temperature range of infection.

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# INHERITANCE OF RESISTANCE TO TOBACCO-MOSAIC DISEASE IN THE PEPPER<sup>1</sup>

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(Accepted for publication March 10, 1937)

Varieties of the garden pepper, *Capsicum frutescens* L., differ in resistance to systemic spread of ordinary tobacco-mosaic virus (tobacco virus 1). Many varieties are characterized by systemic chlorosis when infected. Yellowish primary lesions appear on inoculated leaves, and upon systemic spread of the virus, mottled and distorted leaves are produced. On the other hand, a few varieties, notably Tabasco, are characterized by localized necrosis. Necrotic lesions appear at the site of infection on the second or third day after inoculation. During the next few days the plants lose all infected leaves by abscission, becoming free of virus by this process. The recovered plants are susceptible to reinfection, with repetition of the phenomenon of localization of virus and abscission of infected leaves. In a previous paper,<sup>2</sup> it was shown that a dominant gene (*L*) is characteristic of the localized-necrosis type of pepper, a recessive allele (*l*) taking its place in the systemic-chlorosis or mottling-type varieties.

Earlier study had led to the conclusion that a third kind of response to infection occurred in two varieties of the pepper, Long Red Cayenne and Sunnybrook Cheese, and in occasional plants of several other varieties. It was at first believed that visible symptoms did not develop in plants showing this type of response, because virus was found in uninoculated leaves in which symptoms had not been noted. More careful examination of the leaves, however, showed that a few relatively inconspicuous isolated yellowish lesions, generally becoming wholly or partly necrotic, occurred on young leaves at the top of the plant wherever virus had established itself in measurable concentration. The production of these seminecrotic lesions will be referred to as the delayed-necrosis, or imperfectly localized, type of response. In the present paper, the genetic relationships between the imperfectly localized type of response and the two previously recorded types, systemic chlorosis and localized necrosis, are shown by results from hybridization experiments.

<sup>1</sup> Published at the expense of The Rockefeller Institute for Medical Research, Princeton, N. J., out of the order determined by the date of receipt of the manuscript. This practice in no wise delays the publication of manuscripts printed at the expense of The American Phytopathological Society or other agency.

<sup>2</sup> Holmes, F. O. Inheritance of ability to localize tobacco-mosaic virus. *Phytopath.* 24: 984-1002. 1934.

## EXPERIMENTS WITH LONG RED CAYENNE PEPPER

Plants of *Capsicum frutescens* var. Long Red Cayenne consistently responded to inoculation with *distorting* strain tobacco-mosaic virus<sup>3</sup> either by production of yellowish primary lesions in which there was some necrosis, or by production of large necrotic primary lesions. In most cases these plants escaped systemic necrosis by early abscission of inoculated leaves. When systemic spread of virus occurred, secondary lesions were few. No mottling-type variants were found, nor any plants showing small sharply defined necrotic lesions like those of the previously studied Tabasco pepper.

A typical plant of Long Red Cayenne pepper was crossed with a plant of a mottling-type stock. The  $F_1$  hybrids did not give the seminecrotic response characteristic of the Long Red Cayenne pepper, nor did they mottle. They all showed large necrotic primary lesions, which were followed in most plants by severe systemic necrosis; 60 of 74 infected plants died within a few days as a result of the disease. A few individuals escaped systemic necrosis by early abscission of inoculated leaves, but, upon reinoculation, these plants also succumbed to systemic disease. This typically fatal systemic necrosis differed from both parental responses.

An uninoculated  $F_1$  plant was pollinated from the mottling-type stock, and 146 seedlings were tested by inoculation; of these, 77 showed systemic necrosis like that of the  $F_1$  plants, and 69 showed mottling like that of the mottling-type parent. In this backcross generation, a 1:1 ratio of these two types would indicate that a single partially dominant gene, derived from the Long Red Cayenne pepper, and not present in the mottling-type stock, determined the systemic necrosis in the  $F_1$  plants. The observed ratio of 77:69 is a sufficiently close approximation to a 1:1 ratio to allow a tentative assumption of the presence of such a single gene.

The relation between this newly found gene and the previously known, completely dominant gene *L* was tested by crossing the Long Red Cayenne pepper and an *LL* stock (a mosaic-resistant derivative of California Wonder). All of 109 inoculated  $F_1$  plants showed typical *L*-type necrotic local lesions and recovered by abscission of inoculated leaves. The effect of the gene *L* was thus shown to be completely dominant over that of the gene from the Long Red Cayenne pepper.

After their recovery, several  $F_1$  plants were pollinated from a mottling-type stock. Altogether 505 plants of the progeny were tested by inoculation; 253 of these showed the response expected of heterozygotes bearing one mottling-type gene and one *L*-type gene, and 252 showed the response expected of heterozygotes bearing one mottling-type gene and the gene characteristic of the Long Red Cayenne pepper. If the genes from the Long

<sup>3</sup> Holmes, F. O. Comparison of derivatives from distinctive strains of tobacco-mosaic virus. *Phytopath.* 26: 896-904 (see pp. 897-898). 1936.

Red Cayenne pepper and the gene *L* were in non-homologous chromosomes, or very differently located in homologous chromosomes, many of the gametes produced by the  $F_1$  plants should have lacked both of them, and so should have allowed the formation of corresponding numbers of mottling-type plants. No mottling-type plants were found. It may be concluded, therefore, that the gene from the Long Red Cayenne pepper is either located close to the position of the genes *L* and *l*, or is a member of a series of multiple alleles in which the latter are included. For the purpose of briefly designating the new gene by a symbol, it will be assumed tentatively that it is a member of an allelic series of which previously only *L* (localization of tobacco-mosaic virus), and *l* (mottling) were known. It will be referred to as  $l^1$  (imperfect localization of tobacco-mosaic virus). The data from the experiments with the variety Long Red Cayenne are summarized in the first part of table 1.

#### EXPERIMENTS WITH SUNNYBROOK PEPPER

Although not related to the Long Red Cayenne pepper in any obvious way, the variety Sunnybrook (apparently identical with the Sunnybrook Cheese variety tested earlier) shows consistently a similar type of response to infection with tobacco-mosaic virus. Appropriate crosses were made, as with the variety Long Red Cayenne, and the results are shown in the second part of table 1.

The results of inoculation tests of derivatives of the Sunnybrook pepper were like those obtained with Long Red Cayenne pepper derivatives. In

TABLE 1.—Summary of hybridization experiments with three varieties of *Capsicum frutescens* each characterized by possession of the gene  $l^1$  (imperfect localization of tobacco-mosaic virus)

Genetic constitution of parents	Ratios of plants showing several types of disease symptoms after indicated hybridizations of the following three varieties of pepper		
	Long Red Cayenne	Sunnybrook	Sweet Meat Glory selection
A. $l^1 \times l^1$ .....	188 all of delayed-necrosis type	62, all of delayed-necrosis type	37, all of delayed-necrosis type
B. $l^1 \times l$ .....	74, all of systemic-necrosis type	152, all of systemic-necrosis type	29, all of systemic-necrosis type
C. $(l^1 \times l) \times l$ .....	77 systemic-necrosis type: 69 mottling type	55 systemic-necrosis type: 63 mottling type	142 systemic-necrosis type: 137 mottling type
D. $l^1 \times LL$ .....	109, all of localized-necrosis type	3, all of localized-necrosis type	6, all of localized-necrosis type
E. $(l^1 \times LL) \times l$	253 localized-necrosis type: 252 systemic-necrosis type: 0 mottling type	96 localized-necrosis type: 107 systemic-necrosis type: 0 mottling type	72 localized-necrosis type: 50 systemic-necrosis type: 1 mottling type

the Sunnybrook pepper, therefore, a similar partially dominant gene for imperfect localization of virus is located in the chromosome corresponding to that in which the alleles  $L$  and  $l$  occur. Since it behaves like the gene  $l^1$  of Long Red Cayenne pepper, it is assumed to be identical with this gene, and will be designated by the same symbol. The additional data obtained with these derivatives of the Sunnybrook pepper tend to confirm the conclusion that the gene  $l^1$  is not merely located close to the position of  $L$  but probably should be considered to be an allele of  $L$  and  $l$ , no crossing-over having occurred to give mottling-type plants among 203 individuals of parentage  $Ll^1 \times ll$ .

#### OCCURRENCE OF GENE $l^1$ IN OTHER PEPPER VARIETIES

Among seedlings raised from commercial lots of seeds representing mottling-type varieties, a response like that characteristic of the gene  $l^1$  has been found in one or more plants of each of the following varieties: Anaheim Chili, Magnum Dulce, Red Cluster, Ruby King, Sweet Meat Glory, and Sweet Mountain. The gene  $l^1$  has been sought but not yet found in plants of the following mottling-type varieties: California Wonder, Celestial, Chinese Giant, Coral Gem Bouquet, Early Giant, Giant Crimson, Golden Dawn, Golden Queen, Hungarian, Large Bell, Baby Bell, Oshkosh, Pimiento, Red Cherry, Spanish Monstrous, Upright Sweet Salad, and World Beater. No individuals have ever shown evidence of the presence of the gene  $L$  in tests of the varieties in either of these lists. Since no Tabasco pepper has yet been found to lack this gene, evidence is in hand to show that chance hybridization with the variety Tabasco has been rare or absent. In laboratory tests difficulty has been met in crossing the Tabasco pepper with other varieties, and sterility has been observed in  $F_1$  hybrids having the Tabasco pepper as one parent.

In tests of the variety Sweet Meat Glory, mentioned in the first of the two preceding lists, 49 of 50 inoculated plants mottled; the remaining plant showed some necrosis as a result of inoculation, but later recovered and bore fruit closely resembling that typical of the variety. The response was essentially like that found in the varieties Long Red Cayenne and Sunnybrook. From seed of this exceptional plant, 45 plants were grown. Upon inoculation, none produced mottling symptoms, all, on the contrary, responding like the parent.

One plant of the 45 tested seedlings was saved for hybridization experiments. The results of appropriate tests are summarized in the third part of table 1. These results were essentially like those for corresponding sets of plants derived from Long Red Cayenne and Sunnybrook peppers, except that one plant mottled in an  $Ll^1 \times ll$  set (Table 1, E), in which no mottling-type plant was expected. The occurrence of this plant might be taken as

proof that crossing-over occurs on rare occasions, indicating very close proximity of the loci of the genes, rather than identity of position within the chromosomes. It is possible, however, that the genes may be true alleles, and that a chromosomal loss in this one exceptional plant may have allowed the recessive response to appear where a heterozygous  $U^l$  or  $Ll$  type response should have occurred. It is even possible that the exceptional plant appeared as a contaminant of the seed lot. In spite of the exception, and whatever its explanation may be, it is believed that the genes  $L$ ,  $U^l$  and  $l$  may properly be considered at the present time as members of a series of multiple alleles.

Summarizing the three sets of tests, which involved altogether 2,034 plants, 287 plants of parentage  $U^l \times U^l$  gave only the imperfectly localized response characteristic of their parent plants; 255 plants of parentage  $U^l \times ll$  gave the systemic-necrosis type of response; 543 plants of parentage  $U^l \times ll$  gave the ratio 274 systemic-necrosis type to 269 mottling type, an approximate 1:1 ratio; 118 plants of parentage  $U^l \times LL$  recovered because of complete virus localization and subsequent abscission of inoculated leaves; 831 plants of parentage  $LU^l \times ll$  gave the ratio 421 localized-necrosis type to 409 systemic-necrosis type, an approximate 1:1 ratio, and in addition gave a single exceptional mottling-type plant as discussed in the preceding paragraph.

#### DISCUSSION

Among large-fruited nonpungent peppers, as commercially planted, the commonest genetic constitution is that characterized by the homozygous recessive  $ll$ . Examples of this are the popular varieties California Wonder, World Beater, and Ruby King. After infection in the field, such plants show yellowish primary lesions, early blanching of young leaves, temporary reduction of growth rate, and eventual mottling. Yield of fruit is greatly reduced.

Pepper varieties of constitution  $U^l$ , such as Long Red Cayenne and Sunnybrook, show yellowish primary lesions with some necrosis, subsequently recovering except for scattered secondary lesions in a few plants. Yield of fruits is not much affected.

Large-fruited varieties of  $LL$  type are not yet in use, but strains have been produced and thoroughly tested as to disease resistance. After infection they show necrotic primary lesions only, and their inoculated leaves are soon lost by abscission. Even when grown beside mosaic tobacco plants and repeatedly inoculated by accidental contact with contaminated cultivating implements as well as by rubbing intentionally with extracts of mosaic plants, they remain essentially unharmed, losing only the leaves actually inoculated. Under observed conditions this loss of leaves has proved negli-

gible in effect on yield. The large-fruited, nonpungent strains of pepper bearing in homozygous condition the Tabasco gene for resistance to systemic attack by tobacco-mosaic disease still lack uniformity, and are not yet of proved yielding ability. Selection for uniformity of yield and type is being continued, and it is hoped that the severe effects of occasional heavy outbreaks of tobacco-mosaic disease on peppers may be avoided eventually by the use of varieties resistant to systemic invasion by the causative virus.

#### SUMMARY

All tested varieties of the garden pepper, *Capsicum frutescens* L., have proved susceptible to infection with tobacco-mosaic virus (tobacco virus 1, *distorting* strain). Four types of response to infection have been found. Two of these were known previously: systemic chlorosis and localized necrosis followed by abscission and recovery. A third, described here, is a delayed necrosis, with abscission of affected leaves often allowing plants to escape systemic spread of virus. The fourth is a systemic necrosis, with stem streak and eventual death in all plants. These four responses are controlled by the three genes *L*, *l*<sup>1</sup> and *l*, which form an allelic series. The gene *L* (localization of tobacco-mosaic virus) is completely dominant over *l*<sup>1</sup> (imperfect localization of virus) and *l* (mottling). The gene *l*<sup>1</sup> is partially dominant over *l*. Infected plants of genetic constitution *ll* show systemic chlorosis; *l*<sup>1</sup>*l*<sup>1</sup> delayed necrosis with leaf abscission and recovery in many plants, small numbers of secondary lesions in a few; *l*<sup>1</sup>*l* systemic necrosis in all plants; and *LL*, *Ll* and *Ll*<sup>1</sup> localized necrosis with subsequent recovery.

FROM THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY OF  
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH,  
PRINCETON, NEW JERSEY.

## PHYTOPATHOLOGICAL NOTE

*Echinulation of Chlamydospores and the Pathogenicity of a Previously Undescribed Physiologic Race of Sphacelotheca cruenta.*—In the taxonomic literature dealing with descriptions of *Sphacelotheca sorghi* (Link.) Clint. and *S. cruenta* (Kühn) Potter, the two species have been distinguished macroscopically by the type of membrane surrounding the sori and microscopically according to the type of sterile cells in the sori. Although the chlamydospores of both species have been reported as smooth, it is possible to detect rather fine echinulations on the chlamydospores of *S. cruenta* when they are observed under the oil-immersion objective. The fact that chlamydospores of *S. cruenta* are echinulate was first pointed out by Wang,<sup>1</sup> who made the following statement: "La spore de cette espèce diffère de celle de la précédente [referring to *S. sorghi*] par son exospore finement échinulée." In a study on the genetics and hybridization in these two species,<sup>2,3</sup> the writer has observed the chlamydospores of a great many collections of both *S. sorghi* and *S. cruenta*. In the material that contains the definitely elongated, sterile cells and that has the macroscopic characters of *S. sorghi*, the chlamydospores are smooth, whereas those formed in sori typical of *S. cruenta* are finely echinulate. Evidence has been obtained of segregation of factors governing these spore characters that would lend weight to the above statement by Wang.

As described in a recent paper,<sup>3</sup> a cross was made between a third generation inbred line of *Sphacelotheca sorghi* and a similarly inbred line of *S. cruenta*. Ninety monosporidial lines of the  $F_1$  hybrid were then backcrossed to the recessive parent *S. sorghi*, and evidence was obtained of the segregation of factors governing the type of sori produced and the color of peridia. Although not previously reported, there was segregation in this cross of factors governing chlamydospore markings. Chlamydospores of 85 of the original 90 progeny of the backcross were examined, and of this number 48 were echinulate, while those of the remaining 37 were smooth. Segregation of echinulate and smooth chlamydospores with relation to sorus type in the hybrid progeny was as follows: In 14 of the *S. sorghi* type progeny, 9 had smooth chlamydospores and 5, echinulate; in 49 *S. cruenta* type, 19 had smooth spores and 30, echinulate; in 22 intermediate-type progeny, 10 had smooth chlamydospores and 12, echinulate.

<sup>1</sup> Wang, D. T. Contribution à l'étude des Ustilaginées (Cytologie du parasite et pathologie de la cellule hôte). *Botaniste* 26: 539-672. 1934.

<sup>2</sup> Rodenhiser, H. A. Heterothallism and hybridization in *Sphacelotheca sorghi* and *S. cruenta*. *Jour. Agr. Res. [U. S.]* 45: 287-296. 1932.

<sup>3</sup> Rodenhiser, H. A. Studies on the possible origin of physiologic forms of *Sphacelotheca sorghi* and *S. cruenta*. *Jour. Agr. Res. [U. S.]* (1934) 49: 1069-1086. 1935.  
*lothea sorghi* and *S. cruenta*. *Jour. Agr. Res. [U. S.]* 49: 1069-1086. 1935.

TABLE 1.—Pathogenicity of a previously undescribed physiologic race of *Sphacelotheca cruenta* on the varieties indicated, Arlington Farm, Rosslyn, Va., 1936

Variety	Accession No.	Series 1			Series 2			Average percentage smut
		Total plants	Number smutted	Percentage smut	Total plants	Number smutted	Percentage smut	
Reed kafir .....	C.I. 623 <sup>a</sup>	150	0	0.0	80	0	0.0	0.0
Dwarf Yellow milo .....	C.I. 332	137	0	.0	86	0	.0	.0
Kafir × feterita .....	K.B. 2510	82	69	84.1	79	60	75.9	80.0
Piercee kaferita .....	C.I. 2547	40	0	.0	51	0	.0	.0
Sudan grass .....	F.P.I. 22265	84	15	17.9	79	11	13.9	15.9
Johnson grass .....	—	63	29	46.0	69	28	40.6	43.3

<sup>a</sup> C.I., K.B., and F.P.I. respectively indicate accession numbers of the Division of Cereal Crops and Diseases (formerly Cereal Investigations), the Department of Botany, Kansas Agricultural Experiment Station, and the Division of Plant Exploration and Introduction (formerly Foreign Plant Introduction).



It is commonly observed that in certain collections and physiologic races the echinulations of *Ustilago avenae* (Pers.) Jens. and the reticulations of *Tilletia tritici* (Bjerk.) Wint. vary both in number and size; in fact, such variations have been found between chlamydospores in individual sori. This also holds true for *Sphacelotheca cruenta*. Chlamydospores from infected plants of Johnson grass, collected near Sacaton, Arizona, by H. V. Harlan, had somewhat more prominent echinulations than any of those previously observed. Furthermore, there is strong evidence that this is a physiologic race pathogenically distinct from races 1 and 2 described by Melchers.\* The results of pathogenicity tests are recorded in table 1. These data indicate the susceptibility of kafir  $\times$  feterita and the resistance of Pierce kaferita to this new collection. Melchers\* found both of these varieties susceptible to race 1 and both resistant to race 2. It also should be noted that in the tests at Arlington Farm, Rosslyn, Virginia, no smut developed on Reed kafir. However, in a duplicate test at Kearneysville, West Virginia, in which complete data were not recorded because of poor stands, from 1 to 4 of the lower florets of 7 panicles were found infected.—H. A. RODENHISER, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

\* Melchers, L. E. Physiologic specialization of *Sphacelotheca cruenta* (Kühn) Potter. Jour. Agr. Res. [U. S.] 47: 339-342. 1933.

# REPORT OF THE TWENTY-EIGHTH ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

## THE 1936 ATLANTIC CITY MEETING

The twenty-eighth annual meeting of the American Phytopathological Society, held at Atlantic City, New Jersey (December 28 to 31, 1936), was outstanding. More than 200 members registered. With the election of 139 new members at the meeting the active membership roll reached 965, exceeding all previous records. G. W. Keitt, University of Wisconsin, was elected president; H. W. Anderson, University of Illinois, vice-president, and Charles Chupp, Cornell University, councilor.

**Program.** The scientific program of 99 prepared papers was less congested than in previous years and of high quality. The paper by W. M. Stanley, Rockefeller Institute, presented at the joint session with Section G, "Chemical Studies on the Virus of Tobacco Mosaic", was awarded the \$1,000 A. A. A. S. prize. The presidential address by George H. Coons on "Progress in Plant Pathology: Control of Disease by Resistant Varieties" effectively presented the rôle that heredity, guided by plant pathologists is destined to play in the permanent protection of crops.

The Phytopathologists' Dinner at the Hotel Chelsea, attended by 312 members and guests, will long be remembered for the cleverly worded presentation to the Society, by F. A. Wolf, Duke University, of a curiously wrought gavel, for the unexpected appearance among the diners of raucous newsboys distributing a luridly sensational edition of "The Phytopath Bugle", and for the original and highly entertaining phytopathological stage skit put on by a group of members headed by Wm. H. Martin, R. P. White, and W. H. Weston.

A well-attended joint symposium with the Genetics Society of America, Thursday morning, on "Breeding for Disease Resistance in Plants and Animals" proved highly stimulating. Sessions of interest and value also were held with the Mycological Society of America and the Potato Association of America.

At a conference held Monday evening under the auspices of the Committee on Foreign Plant Diseases and Quarantines energetic discussion and constructive action followed the presentation of papers by Lee A. Strong, Chief of the U. S. Bureau of Entomology and Plant Quarantine; J. F. Adams, Chairman of the National Plant Board, and M. T. Munn, of the Executive Committee of the International Seed Testing Association, which emphasized the need for more adequate provision by this and other nations of biologically sound, effective measures to check the dissemination of destructive plant diseases.

At a round table conducted Tuesday afternoon by the Committee on Coordination of Research and Extension Work, the complexities of the problem of spray and dust injury, and the various types of present-day research related thereto, were informatively discussed.

Concise progress reviews of national campaigns for barberry eradication and for control of the white pine blister rust and the European elm disease were presented by S. B. Fracker and O. N. Liming at the Wednesday afternoon Plant Disease Survey Conference.

**Group conferences.** Efforts of groups within the Society to achieve more effective progress on important problems through mutual assistance and cooperation were advanced by special conferences. The executive committee of the Tobacco Disease Council met on Tuesday evening and that of the Cotton Disease Council on Wednesday evening to plan further coordinated activities. The first group decided to call a general conference of State and Federal workers on the root-knot nematode situation at Nashville, Tennessee, February 2 and 3, 1937. A subcommittee presented for review by the group, and eventual

release to all interested States, a concise, carefully prepared, authoritative statement regarding tobacco viroses and control practices of demonstrated effectiveness in both the plant bed and the field. The other group planned the agenda for the meeting of the Cotton Disease Council scheduled to meet with the Southern Division of the Society at Nashville, February 3-5, 1937.

**Decisions.** The meeting was marked by important decisions and declarations of policy. The Committee on Publication Problems deliberated for nearly 8 consecutive hours on Sunday night, with the Council present by invitation, and formulated proposals later acted on by the Society. The Council and the Society each held 3 official business sessions. The members present pledged themselves to cooperate with the Editorial Board to make conciseness in scientific publication a permanent tradition of the Society and to accept any methods of economy in publication agreed upon as desirable after due study by the Editor in Chief and Business Manager.

The members emphatically opposed the policy of charging authors anything for publication purposes, a Committee on New Memberships and Subscriptions was established. The \$1 per page levy on contributors to PHYTOPATHOLOGY, put into effect the year before, and ordered abstracts of all papers presented at the annual meeting printed hereafter in the journal at the expense of the Society.

To help extend the benefits of membership more widely and to increase income for publication purposes a Committee on New Memberships and Subscriptions was established. The former Committee on Permanent Endowment was replaced by a Committee on Donations and Legacies with broader powers. The complete actions of the Society are itemized at the end of these proceedings.

**Future Meetings.** The Pacific Division was given charge of the summer meeting to be held at Denver, June 20-26, 1937, in connection with the A. A. A. S. An invitation by the Canadian Government to meet in June, 1938, at Ottawa, with the A. A. A. S., was accepted. The next annual meeting will be held at Indianapolis, December 26-29, 1937, according to present plans, and the 1938 meeting in Richmond, Virginia.

#### OFFICERS, REPRESENTATIVES AND COMMITTEES FOR 1937

##### Officers:

- G. W. Keitt, President (1 yr.), University of Wisconsin, Madison, Wis.
- H. W. Anderson, Vice-President (1 yr.), University of Illinois, Urbana, Ill.
- Howard P. Barss, Secretary (3 yrs. Term expires 1937), U. S. Department of Agriculture, Washington, D. C.
- H. A. Edson, Treasurer and Business Manager of PHYTOPATHOLOGY (3 yrs. Term expires 1937), U. S. Department of Agriculture, Washington, D. C.
- H. B. Humphrey, Editor in Chief of PHYTOPATHOLOGY (3 yrs. Term expires 1937), U. S. Department of Agriculture, Washington, D. C.

##### Councillors:

- G. H. Coons (Term expires 1937), U. S. Department of Agriculture, Washington, D. C.
- N. E. Stevens (Term expires 1937), University of Illinois, Urbana, Ill.
- Chas. Chupp (Term expires 1938), Cornell University, Ithaca, N. Y.
- G. F. Weber (1 yr. for the Southern Div.), University of Florida, Gainesville, Fla.
- J. W. Hotson (1 yr. for the Pacific Div.), University of Washington, Seattle, Wash.

##### Representatives:

- A. A. A. S. Council (1 yr.), H. S. Cunningham, W. D. Valleau.

*Elector Group V, Division of Biology and Agriculture, National Research Council* (3 yrs.), E. C. Stakman (H. P. Barss, alternate). (Terms expire 1940.)  
*Board of Governors, Crop Protection Institute* (3 yrs.), J. F. Adams (Term expires 1939), C. R. Orton (Term expires 1938), W. H. Martin (Term expires 1937).  
*Tropical Research Foundation* (5 yrs.), L. R. Jones (Term expires 1940).  
*International Union of Biological Sciences*, Donald Reddick.  
*Board of Editors, American Journal of Botany*, G. W. Keitt (3 yrs. Term expires 1937).  
*Union of American Biological Societies*, Editor in Chief (H. B. Humphrey) and Secretary (H. P. Barss).

#### Standing Committees:

*Foreign Plant Diseases and Quarantines*, C. R. Orton, Chm., H. T. Güssow, J. S. Boyce, W. A. McCubbin, R. D. Rands, J. F. Adams.  
*Extension and Research Coordination*, Chas. Chupp, Chm., R. J. Haskell, A. L. Pierstorff, R. S. Kirby, E. C. Stakman, G. W. Keitt, W. B. Tisdale, I. L. Conners.  
*Coordination in Seed Treatment Research*, C. S. Reddy, Chm., W. E. Brentzel, M. B. Moore, H. A. Rodenhiser.  
*Publication Problems*, H. P. Barss, Chm., H. B. Humphrey, H. A. Edson, R. S. Kirby, E. C. Stakman, N. E. Stevens, M. W. Gardner, R. F. Poole, L. M. Massey, L. R. Jones, F. L. Drayton, G. W. Keitt.  
*Phytopathological Classics*, H. H. Whetzel, Manager; H. B. Humphrey, Editor.  
*Necrology*, A. G. Johnson, Chm., G. P. Clinton, M. B. Waite.  
*Investments*, H. A. Edson, Chm., N. E. Stevens, Chas. Brooks, F. C. Meier, J. W. Roberts.  
*Donations and Legacies*, F. C. Meier, Chm., E. C. Stakman, N. E. Stevens, J. G. Brown, N. J. Giddings.  
*New Memberships and Subscriptions*, R. M. Lindgren, Chm., B. A. Rudolph, Kenneth Kadow, H. P. Barss (ex officio).

#### TEMPORARY COMMITTEES APPOINTED AT ATLANTIC CITY

*Auditing*, Charlotte Elliott, John Monteith, Jr.  
*Elections*, Reiner Bonde, Eldon Lyle.  
*Resolutions*, F. L. Drayton, Anna E. Jenkins, R. P. White.  
*Representatives pro tem., Union of American Biological Societies*, G. W. Keitt, J. G. Brown.  
*Committee on Arrangements, Ottawa Meeting*, H. T. Güssow, Chm., F. L. Drayton, H. B. Humphrey.

#### REPORTS OF OFFICERS, REPRESENTATIVES, AND COMMITTEES FOR 1936

**Report of the Secretary.** The Society year 1936 opened with 832 members and closed with 965, a net gain of 133. At the Atlantic City meeting 139 new members were elected and 29 former members were restored to the active roll during the year. The Society lost 35 members, 8 by resignation, 1 by death, and 26 by suspension for non-payment of dues. Of the full membership, 107 are paid-up life members and 59 are still paying \$10 per year toward life membership. The newly elected members represent 26 states, the District of Columbia, Hawaii, Puerto Rico, Canada, Brazil, Argentina, Ecuador, England, Holland, Norway, Germany, France, Poland, Russia, Algeria, China and Japan.

HOWARD P. BARSS.

**Report of the Treasurer.** Statement of accounts for the year ending November 30, 1936.

*Receipts:*

Balance from 1935 .....		\$2,209.44
Annual dues:		
1934 .....	\$ 20.00 (\$ 10.00 life)	
1935 .....	56.75 ( 10.00 life)	
1936 .....	2,362.06 ( 248.66 life)	
1937 .....	1,869.57 ( 289.90 life)	\$4,313.88
1938 .....	5.00	
Voluntary dues .....		15.00
Items for other accounts included in checks for dues:		
Lyman Fund .....	\$ 10.00	
A. P. S. dinner ticket, 1935 .....	1.75	
Sales .....	2.00	
Abstracts, 1935 .....	14.53	
Publ. of articles in PHYTOPATH. ....	23.00	51.28
Excess, receipts over expenses, A. P. S. dinner, 1935.....		1.60
To replace checks returned by bank .....		12.00
		<hr/>
Total receipts .....		4,393.26
		<hr/>
		\$6,602.70

*Expenditures:*

Member Subscriptions transferred to PHYTOPATHOLOGY		
1934 .....	\$ 12.00	
1935 .....	42.75	
1936 .....	2,955.70	\$3,010.45
Transferred to Sinking Fund (Bldg. and Loan) .....		480.00
Secretarial work for Secretary and Treasurer .....		244.39
Printing and mimeographing .....		193.50
Preprints of abstracts, 1935 .....		27.09
Stamps and stamped envelopes .....		146.19
Supplies .....		2.90
Transferred to PHYTOPATHOLOGY for items in checks		
Abstracts, 1935 .....	\$ 14.53	
Sales .....	2.00	
Publ. of articles .....	23.00	39.53
Transferred to Lyman Fund .....		10.00
Transferred to A. P. S. dinner fund, 1935 .....		1.75
Voluntary dues, transferred to PHYTOPATHOLOGY .....		15.00
Proof-reading Phytopathological Classic .....		21.36
Dues refunded .....		5.00
Telegrams .....		1.00
Checks returned by bank .....		12.00
Collection charges on checks .....		1.00
		<hr/>
Total expenditures .....		4,211.16
Balance on hand .....		2,391.54
		<hr/>
		\$6,602.70

H. A. EDSON.

**Report of the Business Manager of Phytopathology.** Statement of accounts for the year ending November 30, 1936.

*Receipts:*

Balance from 1935 .....		\$ 642.81
Subscriptions:		
1935 .....	\$ 46.60	
1936 .....	3,000.64	
1937 .....	384.53	
1938 .....	5.50	\$3,447.27
Member subscriptions, 1934, 1935 .....		54.75
Member subscriptions, 1936 .....		2,955.70
Voluntary dues .....		15.00
Sales of back numbers .....		556.76
Advertising:		
1935 .....	\$ 186.56	
1936 .....	718.00	904.56
Interest on Sinking Fund:		
First mortgage notes .....	300.00	
Building and Loan .....	183.21	483.21
Interest on Lyman Fund .....		101.88
First mortgage note paid in full .....		500.00
From members for publication of 1935 abstracts .....		148.87
From A. P. S. for preprints of abstracts .....		27.09
From members for publication of papers .....		233.76
Life-sust. dues included in check for article .....		10.00
To replace checks returned by bank .....		12.00
Item credited by bank in error .....		6.55
Total receipts .....		<u>9,457.40</u>
		\$10,100.21

*Expenditures:*

## Printing and distributing PHYTOPATHOLOGY:

Vol. XXV, No. 12 .....	\$ 371.47	
Index to Vol. XXV .....	277.59	\$ 649.06
Vol. XXVI, No. 1 .....	535.24	
No. 2 .....	725.56	
No. 3 .....	474.47	
No. 4 .....	574.39	
No. 5 .....	608.89	
No. 6 .....	533.40	
No. 7 .....	511.27	
No. 8 .....	436.63	
No. 9 .....	535.19	
No. 10 .....	494.85	
No. 11 .....	425.10	5,854.99
Postage .....	598.13	7,102.18
Secretarial work for Editor in Chief .....		378.48
Expenses of office of Editor in Chief .....		30.00
Secretarial work for Business Manager .....		228.38

Secretarial work for Advertising Manager .....	25.00
Commission, Advertising Manager .....	81.16
Expenses of office of Advertising Manager .....	25.54
Stamps and envelopes, Bus. Mgr. & Editor in Chief .....	74.85
Supplies .....	2.05
Printing letterheads .....	8.89
Storage on back volumes .....	48.00
Recovery of copies, May and June, 1933 .....	109.95
Reinvestment of Sinking Fund .....	500.00
Refund on subscriptions and sales .....	16.46
Preprints of 1935 abstracts .....	27.09
Resetting article .....	6.14
Transferred to A. P. S. for life-sust. dues .....	10.00
Checks returned by bank .....	12.00
Debited by bank for item credited in error .....	6.55
<b>Total expenditures .....</b>	<b>8,692.72</b>
<b>Balance on hand .....</b>	<b>1,407.49</b>
	<b>\$10,100.21</b>

*Non-member Subscriptions.* At the end of 1935 there were 568 non-member subscribers to PHYTOPATHOLOGY, including 6 complimentary. During the year 1936 we discontinued one complimentary subscription, there were 42 cancellations and 20 suspensions for non-payment, a loss of 63, but with 87 new subscriptions the net gain is 24, increasing the list at the close of 1936 to 592. Of these, 178 are domestic and 414 foreign. Japan leads with 61, U. S. S. R. has 50, England 37, Canada 27, Germany and India each 21, and China 19. Sixty-nine other countries or geographical units receive from one to 15 copies each.

H. A. EDSON.

*Sinking Fund.* The Sinking Fund, obtained by deducting \$5.00 from each \$10.00 life-membership installment, totaled \$8,511.00 at the close of 1935. During 1936 this amount has increased to \$9,036.00 and is invested as follows:

First-mortgage notes deposited with the McLachlen Banking Corporation for collection, 6% .....	\$4,500.00
Invested with the following building and loan associations:	
Northwestern Savings & Loan .....	1,000.00
National Permanent Building Association .....	500.00
District Building and Loan .....	1,000.00
Columbia Permanent Building Association .....	500.00
Perpetual Building Association .....	1,000.00
Prudential Building Association .....	536.00
	<b>\$9,036.00</b>

*The Lyman Memorial Fund* for the permanent endowment of PHYTOPATHOLOGY.  
Account for the year 1936.

On hand Jan. 1, 1936 (including interest of Dec. 31, 1935) .....	\$2,223.53
Contributions to the fund during the year .....	433.01
Interest on July 1 .....	54.15
	<b>\$2,710.69</b>

Less interest (Dec., 1935, and July, 1936) transferred to PHYTOPATHOLOGY .....	101.88
Balance on hand (not including interest of 12/31/36) .....	\$2,608.81

**Report of the Auditing Committee for the Year Ending November 30, 1936.** The books of the Treasurer of the Society and the Business Manager of PHYTOPATHOLOGY have been examined, together with the present investments of the Sinking Fund and the Lyman Memorial Fund. The accounts have been found correct and the books in excellent order.

December 14, 1936.

CHARLOTTE ELLIOTT, JOHN MONTEITH, JR.

**Report of the Editor in Chief.** PHYTOPATHOLOGY, Volume 26, 1936, contains, exclusive of the index, 1,160 pages of printed text and illustrations (42 pages more than Volume 25), classified as follows: Ninety-five articles, 33 phytopathological notes, 6 reports of regional or other meetings, 7 book reviews, 91 abstracts, 189 text figures and 3 plates. From Jan. 1 to Dec. 31, inclusive, approximately 95 manuscripts of articles, phytopathological notes, reports, book reviews, etc., were submitted for publication in our journal. Of this number 9 major papers and 2 minor ones were returned to their authors for revision, and 2 were withdrawn. Of the manuscripts submitted in 1935, too late for publication that year, 13 were returned in the current year for revision. The index for Volume 26 was published in the December, 1936, number.

At the annual meeting a year ago we were duly authorized to charge contributors to our journal a uniform rate of \$1.00 per printed page of such of their papers as might be accepted and published.

This departure from former practice has proved to be very generally unpopular from the standpoint of our membership and has worked somewhat to the disadvantage of the quality of our journal as an organ of a large guild of world-wide nature and importance. It is, of course, highly desirable, and in our case imperative, that each contributor of a manuscript to PHYTOPATHOLOGY, before submitting it, see to it that his manuscript be a model of conciseness, devoted to a logical presentation of only such matter as is strictly essential to an adequate account of his contribution to our knowledge of the subject that has claimed his interest. The dollar-per-page assessment has in some instances skeletonized manuscripts to their detriment and has reduced too many of them to mere notes. Phytopathological notes are welcome; but is it our desire or aim to sponsor a journal devoted largely to notes? The tendency seems now to be rather decidedly in that direction. The dollar-per-page assessment also has reduced the rate of receipt of acceptable manuscripts, which has fallen from one every 2½ days (1935) to one every 4 days in 1936; and the number of pages has fallen approximately 40 per cent since Jan. 1, 1936.

In ignorance of the actual facts, apparently some have assumed that manuscripts have not received critical examination by others than the Editor before acceptance for publication. The assumption is not wholly warranted, though it is freely admitted that in some cases those to whom the manuscripts were referred should have been more single-minded and severe. It has been and is a part of our editorial policy to submit manuscripts of major articles and sometimes, even of notes to those qualified to examine them and offer constructive criticism. This phase of our editorial practice can and will be extended and improved to the fullest extent practicable. It should be remembered that our editorial service is rendered voluntarily and gratis and most of it is performed, and rightly so, during other than your editor's official hours. Under present circumstances, therefore, it should be the consistent aim of each and every contributor to PHYTOPATHOLOGY to



submit for publication the finished rather than the raw product of his hours of labor and interpretation.

H. B. HUMPHREY.

**Report of the Advertising Manager.** The 1936 income from advertising in PHYTOPATHOLOGY totaled \$980.55, an increase of 7.7 per cent over that of 1935. In fact, advertising in 1936 netted the Society the largest income in the past 5 years. The gross income of 1934 was \$19.90 higher than that of 1936, but 12½ more pages were filled.

One hundred sixty-one pages of advertisements were run in 1936. Of these, 109 or 67.8 per cent were revenue advertisements, occupying 55½ pages. Of these 109, 43 were full-page; 30, ½-page; 24, ¼-page; and 12, ⅓-page advertisements.

The increase of 7.7 per cent in the value of the revenue-producing advertisements, over that of 1935, was accomplished by selling more space for small advertisements and premium space, without increasing the number of pages used.

Fifty-two advertisements, occupying 39½ pages, were non-revenue-producing. These consisted of exchange advertisements with other journals, advertisers' directory, and advertisement of Phytopathological Classics.

In 1936, 19 commercial concerns advertised their merchandise in PHYTOPATHOLOGY.

R. S. KIRBY.

**Report of the Manager of Phytopathological Classics for the fiscal year from December 16, 1935, to December 15, 1936.**

Number of Classic No. 1 on hand Dec. 16, 1935 .....	227
Total number sold .....	7
Balance on hand Dec. 15, 1936 .....	220
Number of Classic No. 2 on hand Dec. 16, 1935 .....	427
Total number sold .....	8
Balance on hand Dec. 15, 1936 .....	419
Number of Classic No. 3 on hand Dec. 16, 1935 .....	555
Total number sold .....	10
Balance on hand Dec. 15, 1936 .....	545
Number of Classic No. 4 on hand Dec. 16, 1935 .....	624
Total number sold .....	13
Number given free .....	2
Total number disposed of .....	15
Balance on hand Dec. 15, 1936 .....	609
Cash balance on hand Dec. 16, 1935 .....	\$382.18
Total receipts from sales for year .....	37.91
Total cash income .....	\$420.09
Expenditures—postage .....	2.00
Balance on hand Dec. 15, 1936 .....	\$418.09
Balances due on account Dec. 15, 1936 .....	3.00

I regret that no new number of the Phytopathological Classics has been issued during the year.

The manuscript of Miss Vera Turin's translation of Nawashin's, "The Sclerotinia of Birch Catkins" has been in my hands for some months. However, due to pressure of a heavy teaching load and other duties, I have been unable to put it into final condition for editorial scrutiny.

No other manuscript has been submitted to me for publication. Funds are available for publication of a Classic of modest size. Members are urged to submit proposals for translations of desirable papers for these Classics. As soon as an acceptable manuscript is available I shall proceed to publish it.

H. H. WHETZEL.

**Report of Committee on Necrology.** During the calendar year 1936, there has been one death, namely, Dr. Ism' A. Hoggan, December 28, 1936.

A. G. JOHNSON.

**Report of the Committee on Permanent Endowment.** The Committee commenced on November 15, 1935, a systematic attempt to increase the Lyman Memorial Endowment for the support of PHYTOPATHOLOGY. On December 31, 1936, before the new Committee on Donations and Legacies took over the work of the Permanent Endowment Committee as part of its responsibilities, a total of \$2,624.81 had been accumulated, not including outstanding pledges or \$105.75 of interest accrued during the past two years, which, by vote of the Society, has been applied to the support of PHYTOPATHOLOGY. Between these dates a total of \$546.50 in cash and pledges had been received as follows: District 1 (Conn., Del., Me., Md., Mass., N. H., N. J., N. Y., Pa., R. I., Vt., W. Va.) no cash or pledges; Dist. 2 (Ala., Ark., N. C., S. C., Fla., Ga., Ky., La., Miss., Tenn., Va.) cash \$10; Dist. 3 (Ill., Ind., Ia., Mich., Mo., O., Wis.) cash \$50; Dist. 4 (Colo., N. D., S. D., Kans., Mont., Neb., Wyo., Minn.) cash \$217, pledges \$43.50 (Editor's note: In this district Minnesota raised \$200 in cash and \$40.00 in pledges); Dist. 5 (Ariz., Cal., N. M., Nev., Okla., Tex., Utah) cash \$130, pledges \$79; Dist. 6 (Ida., Ore., Wash.) cash \$7. Total cash, \$414.00. Total pledges \$132.50.—E. C. STAKMAN, Chm., J. G. BROWN, L. R. HESLER, A. J. RIKER, H. H. WHETZEL.

#### **Report of the Resolutions Committee.**

1. RESOLVED that The American Phytopathological Society express its appreciation to the members of the A. A. A. S. committees responsible for the excellence of the arrangements that have contributed so largely to the success of the 1936 convention at Atlantic City.

2. RESOLVED that The American Phytopathological Society extend its gratitude to the managements of the Ambassador and Chelsea Hotels for the courtesy and efficient service shown to the members attending its 28th annual meeting.

3. RESOLVED that the Society express its thanks to Dr. F. A. Wolf for his presentation to the Society of a gavel, filling a long-felt need. (May our meetings henceforth be conducted with efficiency and dispatch.)

4. In view of the many grave matters facing our Society, and in view of the time and energy expended by our officers and permanent committee members in the solution of these problems:

BE IT RESOLVED, that we in attendance at our 28th annual meeting express our sincere appreciation of their unselfish and untiring efforts in behalf of the Society.

AND BE IT FURTHER RESOLVED that we pledge ourselves, as members, to cooperate in every possible way with our officers and committees throughout the coming year, whenever called upon by them, in the interest of continued progress.

5. RESOLVED that the members of the American Phytopathological Society attending the 28th annual meeting take much pleasure in extending thanks to the entertainment committee composed of Drs. W. H. Martin, R. P. White, and W. H. Weston, Jr. and their troupe, and in expressing their appreciation for the well-cooked mess of pottage served in the form of mirth-provoking antics at our banquet.

**Report of the Representative on the International Union of Biological Sciences.** The Union is in its formative stage. Its potential usefulness is said to have been strengthened by the adherence of the U. S. A. and it is likely that further strength would be added if two or three other countries in which much biological work is done decide to adhere.

How useful the Sub-section for Phytopathology may become depends entirely upon the use made of it. The Union officials do not initiate work; they merely try to execute mandates handed to them by plenary action of competent persons assembled in international congresses.

Your representative endeavors to keep himself informed about events and procedure in order to be of assistance to those groups of the membership that may desire to formulate plans for international action. At the moment his duties do not tax his capacity for such service and until that time arrives he will be able to serve *ex-officio* as a member of such special committees as may be designated for the study of particular problems pertaining to international affairs in the realm of plant pathology.

DONALD REDDICK.

**Report of the Representative of Group V, Division of Biology and Agriculture, National Research Council.** The regular meetings of the Division have been attended. The National Research Council functions in an advisory, stimulative, and coordinative capacity. The Division gives encouragement to and attempts to obtain support for meritorious undertakings of general interest within its field and assists in coordinating, largely through the activities of special committees, the efforts of various agencies working on related problems.

During the year 1935-1936 appeared two important volumes on the Biological Effects of Radiation edited by B. M. Duggar, member of this Society, as chairman of the Committee on Radiation. Of major concern to the Division has been the problem of future support for Biological Abstracts, on which a special committee has been working. A Committee on Apparatus was authorized to promote the loan, exchange, or cooperative use, of special apparatus and to provide information to research workers or agencies as to where special or unusual apparatus, desired for particular purposes, might be obtained.

In March, 1936, 22 new appointments were made to National Research Fellowships in the Biological Sciences. Continuance of these fellowships for the triennium beginning next July has been assured by a grant from the Rockefeller Foundation. Twelve workers in biology received grants-in-aid in 1935-36, one grant for work on substances promoting reproduction in fungi being assigned to a member of this Society, L. H. Leonian. The funds that supported these grants-in-aid have now been exhausted and no further grants will be made unless other funds be made available.

The Society's representative was elected Vice-Chairman of the Division of Biology and Agriculture for 1936-1937 and E. C. Stakman, member of the Society, was elected Member-at-Large on the Executive Committee of the Division for the biennium 1936-1938. At the Atlantic City meeting the Society elected E. C. Stakman, with H. P. Barss as alternate, to serve as elector for Group V of the Division to choose a representative on the Division for 3 years beginning July 1, 1937. The representative will be selected this time from the membership of the Society of American Bacteriologists.

HOWARD P. BARSS.

**Report of Representatives on the Board of Governors, Crop Protection Institute, for 1936.** The personnel of the Board of Governors for the year was as follows: W. C. O'Kane, Chairman, Durham, New Hampshire; W. P. Flint, Urbana, Illinois; C. H. Richardson, Ames, Iowa; W. H. Martin, New Brunswick, New Jersey; C. R. Orton, Morgantown, West Virginia; J. F. Adams, Vice-Chairman, Newark, Delaware; H. J. Patterson, College Park, Maryland; W. H. MacIntire, Knoxville, Tennessee, and R. Kellogg, Washington, D. C., representing the American Association of Economic Entomologists, The American Phytopathological Society, The Association of Official Agricultural Chemists, and The National Research Council, respectively.

Sixteen projects were maintained during 1936, including exploratory or preliminary studies, as well as major full-time projects. Eighteen research men were employed, on a full-time or part-time basis. Eight were engaged in work relating largely to entomology, 9 on problems in plant pathology and 1 on problems of animal diseases. Research projects or related field work were established in California, Colorado, Delaware, Illinois, Indiana, Iowa, Louisiana, Maryland, Massachusetts, Missouri, New Hampshire, New Jersey, New York, North Carolina, Ohio, South Carolina, and Virginia.

The research projects conducted in 1936 included: Developing new copper fungicides, copper sulphate as a soil amendment, pyrethrum propagation, synthetic organic compounds as fungicides and insecticides, substitute insecticides for lead arsenate, performance of calcium arsenate, cuprous oxide as a fungicide, pyrethrum and nicotine sprays, spreading and wetting agents, iodine compounds and control of coecidiosis.

Full or part-time projects were sponsored by the following companies: Nichols Copper Company, Stanco, Inc., Mansanto Chemical Co., Dow Chemical Co., National Aniline Co., General Chemical Co., Metals Refining Co., Röhm and Haas Co., General Dyestuff Corp., Amino Products Co., Rare Chemicals, Inc., and Standard Chemical Products, Inc.

The following were published in the Institute bulletin series in 1936:

- No. 52, Sulfuric Acid for Control of Weeds, by W. E. Ball and O. C. French.
- No. 54, The Role of Pine Oil in Cattle Fly Sprays, by Allen M. Pearson.
- No. 55, Copper Sulfate as a Plant Nutrient and Soil Amendment, by W. L. Churchman, R. Russell and T. F. Manns.
- No. 56, The Crop Protection Institute, Its Organization, Plan of Procedure, and Work Accomplished, by W. C. O'Kane.
- No. 57, Ovicidal and Scalecidal Properties of Solutions of Dinitro-O-Cyclohexylphenol in Petroleum Oil, by J. Franklin Kagy and Charles H. Richardson.
- No. 58, Toxicity of Some Nitro-Phenols as Stomach Poisons for Several Species of Insects, by J. Franklin Kagy.
- No. 59, Laboratory Method of Comparing the Toxicity of Substances to San Jose Scale, by J. Franklin Kagy.
- No. 60, Halowax (Chlorinated Naphthalene) as an Ovicide for Codling Moth and Oriental Fruit Moth, by E. P. Breakey and A. C. Miller.

J. F. ADAMS.

**Report of the Committee on Publication Problems.** With the assistance of many other members, the Committee has made a careful study of the situation relating to the publication problems that have confronted the Society. Suggestions as to ways and means of meeting these problems have been received, compiled, and distributed among the members of the Committee and the Council. Careful estimates of the costs of operation and the present and prospective income of PHYTOPATHOLOGY were prepared and distributed in the same manner. Conferences were held with publishers of the Journal and with various authorities on printing and publication problems.

The Committee met Sunday evening, December 27, 1936, with the majority of the Council, reviewed the entire situation and prepared ten recommendations for presentation to the Council. The Council ordered them presented directly to the Society for consideration, which was done at the business meeting on Monday, December 28. Action was postponed until the business meeting on Wednesday, December 30, to give ample opportunity for consideration by the membership. Since these proposals will be listed under the actions taken by the Society, they will not be presented here.

The Committee suggests that it be continued to carry on its studies and make such further recommendations as may seem desirable.

HOWARD P. BARSS.

**Report of the Committee on Foreign Plant Diseases and Quarantines.** Pursuant to the actions taken by the Society at St. Louis, January 3, 1936, your committee presented in person to the Honorable Secretary of Agriculture, the Chief of the Bureau of Plant Industry, and to the Chief of the Bureau of Entomology and Plant Quarantine the resolutions passed at that meeting. These included:

1. A consideration of more adequate detention services.
2. A continuation and expansion of policy of sending specialists into foreign countries to study potentially dangerous diseases.
3. A warning regarding the introduction of biological races.
4. The entry of living cultures of plant pathogens only under permit.
5. The extension of European Elm Disease Survey.
6. A request that the Bureau of Entomology and Plant Quarantine consider further the desirability of carrying out eradication of the potato wart disease.
7. A suggestion that the Council of The League of Nations be requested to arrange for the discussion of quarantines and related questions.
8. The recommendation that The American Phytopathological Society arrange for a discussion of quarantine problems at the next annual meeting.

The presentation of these resolutions brought response from the Bureau of Entomology and Plant Quarantine regarding the entry of living cultures, the potato-wart situation, and the European Elm Disease. In these communications, the inconsistency of allowing living cultures of pathogens to enter without control from foreign countries was acknowledged. The resolution regarding the potato-wart disease brought prompt action in the form of a special study and report from the Bureau. Plans are being considered for an eradication program. With respect to the expansion of the European Elm Disease Survey to include all outlying districts, it appears that this is dependent upon additional funds being made available.

Your Committee has arranged a program for the Atlantic City meeting on Monday evening, December 28, in the Auditorium. It is believed that this meeting will be helpful in further acquainting our Society members with the problem concerning quarantines and foreign plant diseases.

C. R. ORTON.

**Report of the Committee on Coordination of Extension and Research.** Through the Secretary of the Society copies of the two resolutions relating to the more effective coordination of research and extension that were passed at the St. Louis meeting were sent to all directors of research and extension and to all heads of plant pathology departments in State institutions, as well as to certain Government officials. The numerous replies received indicated a willingness on the part of directors to cooperate in worthwhile efforts for joint attack on regional plant disease control problems.

The Committee made arrangements for, and conducted a round-table conference on the subject of spray and dust injury at the Atlantic City meeting. A summary of results of a questionnaire on lime sulphur injury sent to all States and certain Canadian Provinces was distributed in mimeographed form.

CHAS. CHUPP.

**Report of the Committee on Coordination of Potato Disease Research.** In 1936 efforts were continued to encourage plant pathologists to take a more active part in the national program of potato improvement. The Committee aided in making arrangements for a joint conference of potato breeders and pathologists, held in Iowa, Minnesota, and North Dakota the week of August 24-29, inclusive, 1936. The Committee issued invitations to potato pathologists of all of the several experiment stations of the country. It also sent a letter to the directors of the experiment stations informing them of the conference. At the conclusion of the conference, the Committee made mimeographed copies of the report and distributed them to interested parties, including the directors of the experiment stations.

The conference was successful, and was attended by 14 horticulturists, 12 plant breeders, and 10 plant pathologists. It was the opinion of those in attendance that, "the 1936 conference of potato breeders and pathologists has been particularly successful in fulfilling the expectations of those who proposed the conference. The possibilities which they felt existed for the furthering of cooperation among the potato research workers and coordination of the work have become actualities. Every person attending this conference is better acquainted with his fellow worker, is more conversant with the problems of each particular potato section, and can visualize more clearly the national potato problem as a whole. . . . Every one present heartily endorsed the resolution that a similar group should meet next year."

The Committee feels that it has largely accomplished the objectives for which it was appointed, namely, to stimulate greater interest of the pathologists in the potato-improvement program and to bring about better coordination of the pathological and plant breeding phases of the potato improvement work. The Committee respectfully requests that it be discharged.

J. G. LEACH, Chm., R. W. GOSS, J. C. WALKER, D. REDDICK.

**Report of the Committee on Coordination of Seed Treatment Research.** In March, 1936, the Committee distributed outlines for uniform tests on wheat, oats, corn, barley, and flax seed treatments for use by States interested in conducting such work and in comparing results. The drouth and other unfavorable conditions so interfered with the tests that the season's results proved unsatisfactory in many cases. It is hoped that further cooperation along similar lines may be more successfully conducted in 1937. The Committee is continuing its plan to carry forward the group program.

C. S. REDDY.

**Report of the Tobacco Disease Council.** The second annual conference of the Tobacco Disease Council was held June 24 to 26, 1936, at Tifton, Georgia, and Quincy, Florida. Twenty-six workers attended, representing 9 States, the U. S. Department of Agriculture and the Canadian Government.

Special subcommittees were formed to sponsor and coordinate various lines of work as follows: 1. On stem and root diseases, R. F. Poole, Chm., 2. on virus diseases, W. D. Valleau, Chm., 3. on leaf diseases, E. E. Clayton, Chm., 4. on tobacco disease survey, Luther Shaw, Chm., 5. on tobacco insects, W. D. Reed, Chm.

The subcommittees are (1) to define work objectives with respect to specific diseases, (2) to aid individual members in conducting their work and to offer opportunities for better-coordinated effort, and (3) to present progress reports at the annual meetings.

The chairman of the subcommittees automatically become members of the Executive Committee, which is now composed of the following: S. A. Wingard, Chm., R. G. Henderson, Secretary, R. F. Poole, W. D. Valleau, E. E. Clayton, G. M. Armstrong, Luther Shaw, and W. D. Reed.

A very instructive program was presented on the nematode research in southern Georgia. The experimental plots at the Coastal Plains Station were visited and results explained by Mr. J. G. Gaines. The North Florida Experiment Station also was visited and the work there on black shank explained by Doctors Gratz and Kincaid. During the meeting progress reports were made on other tobacco-disease research.

The proceedings of the meeting were mimeographed and distributed to the members. Outlines of all the projects on tobacco diseases now under investigation by members have been supplied to each institution represented by the Council.

R. G. HENDERSON.

**Report of the Cotton Disease Council.** A conference for coordination of cotton-disease research was held at Jackson, Mississippi, February 6-7, 1936. As a result the Cotton Disease Council, a permanent organization, was formed with membership open to all plant pathologists concerned with cotton diseases. The conference was devoted to (1) discussions of the present status of knowledge and the further studies needed relative to the more important cotton-disease problems and (2) the organization of the Council and adoption of plans for its work. The following Executive Committee of 6 members was chosen: G. M. Armstrong, Chairman, H. D. Barker, L. E. Miles, C. D. Sherbakoff, J. J. Taubenhau, and V. H. Young. Special subcommittees were established to include all persons working on a specific disease problem. These committees are: Fusarium Wilt, Seed-borne and Seedling Diseases, Verticillium Wilt, Texas Root Rot, and the Cotton Disease Survey. Other committees probably will be established later.

The Executive Committee was given the responsibilities of: (1) The preparation of an outline of the general cotton-disease situation and listing of problems needing attention; (2) The annual distribution to each cooperating institution of sets of the detailed project outlines covering current cotton-disease research submitted by the different institutions and agencies; (3) Assisting in the formation of regional subcommittees; (4) Promoting the exchange of information regarding projects having to do with cotton between plant pathologists, agronomists, chemists and others; (5) Considering the possibilities of cooperative regional use of special equipment or trained personnel at particular institutions and (6) Arranging for the annual conferences, preparing programs for them and distributing the proceedings.

Mimeographed proceedings of the last conference were sent to all those in attendance, and the next conference has been called for February 3, 4 and 5, 1937, at Nashville, Tennessee, in connection with the meeting of the Southern Agricultural Workers and the Southern Division of this Society.

G. M. ARMSTRONG.

#### ACTION BY THE SOCIETY AT THE 1936 ATLANTIC CITY MEETING

**Appointments and Elections.** The appointments made by the President and Council since the previous meeting were approved as given in the list of Officers, Representatives, and Committees appearing in this report. The election ballots for officers were tallied by the Election Committee and canvassed by the Council before the resulting elections were presented to the Society. By unanimous ballot 139 applicants were elected to membership.

**Reports.** The reports printed in these proceedings were accepted as presented with the exception of the reports covering coordination activities, which were ordered printed as a matter of record, without reading.

**Special Resolutions.** These were prepared by the Committee on Foreign Plant Diseases and Quarantines by order of the Monday night conference and were adopted by vote of the Society at the Wednesday business meeting. D. Reddick asked that his opposition to the passing by the Society of resolutions relating to governmental action be made a matter of record.

1. *Resolution Endorsing Establishment by Congress of Emergency Fund Against Plant Disease or Insect Pest Outbreaks.* On recommendation of the Committee on Foreign Plant Diseases, The American Phytopathological Society strongly endorses the resolution passed on November 5, 1936, by the National Plant Board, and attached hereto, urging the necessity in the interest of American agriculture and forestry for the establishment by the Government of the United States of an emergency fund for immediate action against unforeseen epidemic outbreaks of insect pests and plant diseases to become available when any such emergency arises.

Furthermore, this Society hereby respectfully requests the Council of the American Association for the Advancement of Science to endorse this recommendation and officially transmit it to the Honorable Secretary of Agriculture.

*National Plant Board Resolution for an Emergency Fund, Passed November 5, 1936*

Whereas, emergency Federal appropriations for aid in the control of regional epidemic outbreaks of insects usually become available too late seasonally to be expended and used with maximum efficiency and economy, therefore be it

Resolved, that the National Plant Board urge that the 1937 Congress establish, and subsequent Congresses maintain, a fund of five million dollars (\$5,000,000) to be replenished to the original amount at the beginning of each fiscal year whenever such replenishment is necessary, to be available to and administered by the Bureau of Entomology and Plant Quarantine of the United States Department of Agriculture for the financing through advance options, or storage, and subsequent distribution or purchase and distribution at the beginning and during the emergency to the affected States, through cooperation with the proper officials of the States concerned and upon adequate set-off commitments of these States, of insecticides, fungicides, eradicant chemicals, and other useable materials and supplies, equipment, transportation, distribution, and application of materials, administrative costs, salaries, and other incidental and miscellaneous costs and expenses connected with and deemed necessary to accomplish the control of grasshoppers, chinch bugs, and other insect or plant-disease pests, similarly subject to interstate migratory movements and dissemination, or intermittent regional outbreaks affecting several States, or emergency insect or plant-disease epidemic outbreaks that threaten the agricultural interests of several States, or the eradication of new or reintroduced pests not known to occur or not widely distributed in the United States, if and when the need and desirability for such Federal Aid is satisfactorily established through the approval of competent technical committees, the Secretary of Agriculture, the Director of the Budget, and other proper Government agencies.

2. *Resolution Urging Funds for a Program for the Eradication of the Potato Wart Disease.* On recommendation of the Committee on Foreign Plant Diseases, the American Phytopathological Society urges that steps be no longer delayed to secure the necessary funds for the eradication of potato wart disease based upon studies made by the Bureau of Entomology and Plant Quarantine, showing the thorough practicability of complete eradication of this disease.



3. *Resolution Urging Funds for Adequate Study of Azalea Flower Blight.*

Whereas, it has been determined that a serious disease, known as azalea flower blight, threatens to destroy the ornamental value of these shrubs in the South Atlantic States and to spread into other sections of the country, and whereas, adequate researches have not been conducted into the nature and control of this disease, The American Phytopathological Society strongly urges the further allotment of funds for the above purpose to provide the proper biological basis for any legislative action that may become necessary.

**Committee on Seed-borne Diseases in Relation to Possible Plant Quarantine Measures.** The appointment of this subcommittee by the Committee on Foreign Plant Diseases and Quarantines was recommended by the Monday night conference and approved by the Society at the Wednesday business meeting. The following committee was selected to study this subject with the expectation that it would have at least a preliminary report for the next annual meeting: M. T. Munn, Chm., Edgar Brown, G. A. Scott.

**Life Membership Provision Repealed.** The proposal for this amendment repealing Article III, Section 2, of the Constitution prescribing the requirements for life members and donors was sent out to the membership in advance of the meeting. The amendment was passed by a vote of 66 to 2. Only regular, annually-paying memberships are now provided for. Life memberships under the former plan had resulted in losses to the Society and journal in recent years.

**Amendment to be Submitted.** On recommendation of the Council, the Society agreed to submit for vote at the next annual meeting an amendment to the Constitution, Article V, changing the last sentence in the second paragraph to read as follows: "The term of service of a Council member from a Division shall commence immediately following his election and shall continue until his successor is elected."

**Method of Nomination Referred to Council.** The proposal of a member to amend the Constitution so that nominations for officers, three for each office, would be made by a nominating committee of past presidents named by the Council (with opportunity afforded on the ballot for a member to vote, if desired, for other persons than those listed) was referred on motion to the Council for recommendation to the Society as it might see fit.

**Denver Meeting.** On Council recommendation it was voted to place arrangements for the meeting in Denver, the week of June 20, 1937, in the hands of the Pacific Division.

**Ottawa Meeting.** On Council recommendation it was voted to accept the invitation of the Canadian Government to meet in June, 1938, at Ottawa in connection with the summer meeting of the A. A. A. S. with the following Committee on Arrangements: H. T. Güssow, Chm., F. L. Drayton, H. B. Humphrey.

**Centraalbureau voor Schimmelcultures, Baarn, Holland.** On recommendation of the Council, it was voted that the Society become a donor to this valuable international institution, threatened with insufficient income to continue its work. On request of the Council, E. C. Stakman explained the situation, urging members and institutions to aid both by becoming donors and by making more liberal use of this great collection of living cultures of fungi and bacteria.

**Biologists' Smoker.** On recommendation of the Council, it was voted to contribute \$10 to the smoker arranged by the American Society of Naturalists at the Atlantic City meeting.

**Special Committee.** On Council recommendation it was voted that H. T. Güssow, Carl Hartley, and H. P. Barss prepare a reply to a letter from the Committee on Wild Life and Nature Reserves, Division of Biology and Agriculture, National Research Council, calling attention to the need of support for all efforts looking to the conservation of

vegetal resources as fundamental to the maintenance of wild life in America, with special reference to the hazards created by introduced plant diseases and pests.

**Biological Abstracts.** It was voted that the special committee appointed by the Society to represent it at the Monday evening meeting of the Union of American Biological Societies, consisting of G. W. Keitt and J. G. Brown, prepare a letter to the Union expressing the Society's appreciation of the invaluable service rendered by Biological Abstracts and indicating the position of the Society in respect to the suggestions made by the Union.

**Committee on Coordination of Potato Disease Research.** The Society adopted the report of this committee and by so doing accepted the recommendation of the Committee that it be discharged in view of the fact that the major objectives for which it was created were in sight, and better coordination had been achieved between potato pathologists and the potato-breeding program.

#### ACTION OF THE SOCIETY RELATIVE TO PUBLICATION MATTERS

The items below marked (Com.) were submitted to the membership at the request of the Council by the Committee on Publication Problems at the business meeting on Monday and were acted on at the Wednesday business meeting at which time the other motions here included were presented from the floor.

**Committee on New Memberships and Subscriptions. (Com.)** It was voted that the Council appoint a standing committee of 3 members with the Secretary as an additional *ex-officio* member to develop a program for increased memberships and subscriptions to PHYTOPATHOLOGY in a systematic way. The Council appointed the following: R. M. Lindgren, Chm., B. A. Rudolph, Kenneth Kadow, H. P. Barss (*ex-officio*).

**Committee on Donations and Legacies. (Com.)** It was voted that the Council appoint a standing committee (replacing the former Committee on Permanent Endowment) to stimulate contributions to the Society, including contributions to the permanent endowment (Lyman Memorial Fund) and to the current publication expense fund. The Council appointed the following: F. C. Meier, Chm., E. C. Stakman, N. E. Stevens, J. G. Brown, N. J. Giddings.

**Pledge to Support Editorial Board in a Firm Policy. (Com.)** It was unanimously voted that, recognizing the fact that conciseness is essential in the interest of economy and the maintenance of a high standard for the journal, the Society pledges itself to cooperate with the Editorial Board in the attainment of this end and, therefore, authorizes the Editorial Board to take the necessary steps to accomplish it.

**Appreciation of Work of Editor in Chief.** It was unanimously voted that the Society express to H. B. Humphrey its high appreciation of his work as Editor in Chief and pledge its sympathetic support and cooperation in carrying out the added future responsibilities placed on him and his editorial associates at this meeting.

**Printing Economies Authorized. (Com.)** It was voted unanimously that the Editor in Chief and the Business Manager be empowered by the Society to investigate and put into effect such economies in the structure of PHYTOPATHOLOGY as they may deem wise. This motion was accompanied by the following statement: In this connection the Committee desires to emphasize that there is reason to believe that if such economies are effected, if the members of the Society fully cooperate in the condensation of articles, and if contributions come in at the usual rate, it will be possible for PHYTOPATHOLOGY to publish all acceptable articles with reasonable promptness. On the other hand, if submitted material is not effectively condensed or if the number of contributions is greatly increased, it will be absolutely necessary for the Society to seek other sources of revenue, such as increased dues, increased prices for reprints, etc., or again to face serious delays in publication.

**Out-of-Order Publication Continued. (Com.)** It was voted unanimously that the policy of publishing out-of-order papers paid for in full by the contributor or supporting agency be continued.

**Levy on Contributors Revoked. (Com.)** It was voted unanimously that effective December 31, 1936, the Council revoke the \$1 per page levy on contributors, which had been applied to all manuscripts submitted after January 1, 1936.

**Publication of Proceedings and Notices at the Expense of the Society.** It was voted unanimously that the Treasury of the Society meet the cost of publication of all Society proceedings and notices.

**Publication of Abstracts Authorized. (Com.)** It was voted that the Society resume the former practice of publishing in PHYTOPATHOLOGY, at the expense of the Society, abstracts of all papers presented at the annual meeting, such abstracts to be considered as a part of the proceedings. (The sense of this motion was against any charge to authors individually.)

**Action of 1932 Rescinded.** It was voted unanimously that the two acts passed at the Atlantic City meeting in 1932 enabling the Council to place levies on contributors of articles and abstracts be rescinded.

**Publication of Abstracts of the 1936 Atlantic City Meeting.** President Coons ruled that, since it was clearly the wish of the Society that abstracts of papers presented at the meetings be published in PHYTOPATHOLOGY, those presented at the present meeting should be so published, the matter of meeting the expense in this case being left to the officers to handle as they deemed best.

**Publication of Résumés on Plant Disease Control.** Discussion of this proposal by the Committee revealed the fact that as far as any one present knew, the Society had never enacted any restrictions on the character of material published in PHYTOPATHOLOGY. It was held that the Editorial Board had the power of discrimination in such matters. The proposal was, therefore, referred by President Coons to the Editorial Board.

**Publication Problems Committee Continued. (Com.)** It was voted unanimously that the Committee on Publication Problems be continued.

## ISMÉ ALDYTH HOGGAN

March 23, 1899–December 28, 1936

Ismé Aldyth Hoggan graduated from Cambridge University in 1922 with the degree of Bachelor of Science. Under a fellowship from her own college (Newnham) and in a research relation with the Botany School of Cambridge, she continued her studies there for two additional years, securing the Master's Degree in Science in 1927.

Shortly after coming to Wisconsin, Miss Hoggan became a part-time assistant, working on the cytology of plant virus diseases. These earlier and temporary research positions under the Wisconsin Research Fund and the Bureau of Plant Industry, United States Department of Agriculture, developed in 1927 into a permanent relation with the Wisconsin Agricultural Experiment Station and the United States Department of Agriculture cooperating. This position permitted her to utilize almost her entire time on the plant viruses, a subject in which she was greatly interested. Dr. Hoggan was promoted from Instructor to Assistant Professor in the University of Wisconsin in 1933, which position, together with that of Agent, Bureau of Plant Industry, she held at the time of her death.

Dr. Hoggan contributed fifteen technical papers to the field of plant pathology, all but two being on plant viruses. She performed her work and wrote her papers with such painstaking care and accuracy that many of her contributions are worthy models in phytopathological research. Her natural ability and early basic training accounted for the precision in her research and the skill used in interpreting and presenting her results in writing. Ismé Hoggan's colleagues in England and America will long remember her keen intellect and fine, reserved personality.

# ERRATA, Volume 26

- Page 45, line 11, *read* by *for* of.
- Page 51, line 41, *read* (13) *for* (5).
- Page 53, line 19, *read* above, American *for* above. American.
- Page 472, line 38, *read* lends *for* leads.
- Page 493, line 13, *read* Tribolium *for* Tribelium.
- Page 559, line 4, *read* page 552 *for* page 561.
- Page 560, line 39, *read* page 552 *for* page 561.
- Page 639, heading, table 6, *read* spore-infested *for* spore-infected.
- Page 764, line 17, *read* Basidiophora *for* Basidiosphora.
- Page 856, table 8, heading of column 4, *read* Total number of spores caught on 7.5 cm.<sup>2</sup> of slide *for* Total number of spores per cm.<sup>2</sup> per hour.
- Page 838, line 13, *read* table 2 *for* table 4.
- Page 889, line 22, *read* now *for* not.
- Page 993, line 29, *read* in ability *for* inability.
- Page 1046, line 22, *read* Alternaria *for* Altenaria.
- Page 1157, line 35, *read* nomenclature, and painting on the 30th day *for* nomenclature and painting, on the 30th day.



A NEMATOSIS OF SWEET POTATOES CAUSED BY  
*ANGUILLULINA DIPSACI*,<sup>1</sup> THE STEM  
OR BULB NEMA

HANS A. KREIS

(Accepted for publication Feb. 10, 1937)

INTRODUCTION<sup>2</sup>

Early in the spring of 1930 sweet potatoes (*Ipomoea batatas* (L.) Lam.) from some storage houses in New Jersey were reported to be attacked by *Anguillulina dipsaci* (Kühn), the bulb or stem nema.<sup>3</sup> The pest was discovered when seed tubers failed to sprout. Since *A. dipsaci* is known as a serious parasite of some 250 different plant species, many of great economic importance, its discovery on seed sweet-potato tubers was rather alarming. Diseased material was at that time received from Mullica Hill and a section of Vineland, New Jersey, and from Snow Hill in eastern Maryland.

The investigations here reported cover the study of the parasite, its host relationship, probable origin, present distribution, and possibilities for its control. A number of host-transfer experiments were made and the sweet-potato regions of New Jersey, Maryland, Delaware, Virginia, North Carolina, and South Carolina were visited. Later in the season a survey of some storage houses in Maryland and New Jersey was made.

During these investigations not only *Anguillulina dipsaci* but a number of other nemas were found variously associated with sweet-potato tubers—species of the genera: *Acrobeles*, *Aphelenchus*, *Aphelenchoides*, *Heterodera*, *Cephalobus*, *Diplogaster*, *Diploscapter*, *Dorylaimus*, *Criconema*, *Macrolaimus*, *Mermis*, *Mononchus*, *Plectus*, and *Rhabditis*. In the present paper, however, only *Anguillulina dipsaci* will be considered.

Hitherto, only *Heterodera marioni* (Cornu) (= *Heterodera radicicola*) (= *Caconema radicicola*) had been reported as a nemic pest of sweet potatoes (7, 19, 20).

Unfortunately, the time for this study was restricted, and it necessitated a limitation of experiments and observations. This may explain the rather preliminary character of some of the results presented.

<sup>1</sup> Since the preparation of this manuscript the species *Anguillulina dipsaci* has been made the type of a new genus. Its name is now *Ditylenchus dipsaci* (Kühn) Filipjev. See Filipjev, I. N. On the classification of the Tylenchinae. Proc. Helminthol. Soc. Washington 3: 80–82. 1936.

<sup>2</sup> The author wishes to express his appreciation to the Rockefeller Foundation for the grant of a fellowship which made possible the present studies, and the two trips to the eastern sweet-potato regions in the United States. He is also indebted to the Bureau of Plant Industry, U. S. Dept. of Agriculture, for its hospitality and to the late Dr. N. A. Cobb, Dr. G. Steiner and Dr. J. M. Schaffer, of this Department, for their varied assistance.

<sup>3</sup> Steiner, G. Sweet potatoes attacked by *Tylenchus dipsaci*, the bulb or stem nema. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rptr. 14: 109. 1930. [Mimeographed.]

## GENERAL

Sweet potatoes are grown over the entire eastern part of the United States as far north as New Jersey. According to reports in the Yearbook of the Department of Agriculture (28, table 270, p. 778) the whole crop in the United States in 1929 was valued at about \$80,000,000.

During the course of the investigations herein described it was found that all tubers with black, dry, soft, or stem rot also contained nemas. In only one case was *Anguillulina dipsaci* observed in the field, namely at the Coastal Plain Station, Wallace, North Carolina, where the parasite was associated with *Heterodera marioni*.

In the course of field observations it was noted that a sandy soil is more favorable to the development of nemas than a heavy one. Certain nemas gradually disappear from a plant when severe rot sets in. Tubers filled with fungus mycelium usually harbor few or no nemas, even though in the early stages of the disease nematodes had been numerous. Saprophytic nemas use all kinds of lesions to enter a tuber, often bringing with them fungi and bacteria.

The fungi usually develop much more rapidly than do the nemas; hence it is difficult to decide which agent came first, especially since certain forms of nematodes may be repelled by fungus growth and leave a tuber thus attacked. Usually, no fungi are present in sweet potatoes in the early stages of the *Anguillulina dipsaci* disease. The relationship between nemas and plant diseases is perhaps more complicated than is generally suspected.

This paper deals with the symptoms of the *Anguillulina dipsaci* disease of sweet potatoes; host transfer experiments with this form; the use of disinfectants for its control; and suitable seed sweet-potato storage to prevent the disease. Seventy localities were visited on a survey of various sweet-potato sections. This survey was made mainly to determine the presence or absence of *A. dipsaci*. Nemas belonging to the aforementioned genera were found and only in one instance, as mentioned above, was *A. dipsaci* observed in the field, i.e., at Wallace, North Carolina, and then associated with *Heterodera marioni*. In 6 instances at Monroeville, Ewan, and Vineland, New Jersey, and Snow Hill, Maryland, seed sweet potatoes of 1930 in storage were found infested with *A. dipsaci*.

## ANGUILLULINA DIPSACI

Morphologically *Anguillulina dipsaci* has been adequately described by Kühn (15), Debray and Maupas (6), Ritzema Bos (23), Marcinowski (17), Goodey (9), and de Bruyn Ouboter (2). However, since the question of host strains or races in this form often is discussed, rather extensive measurements were made to see if differences in size or of location and relationship of various organs would support the existence of such races or strains.



Specimens from sweet potatoes grown in New Jersey had the following dimensions:

A. Average of 100 females.

Expressed in de Man's<sup>4</sup> formula:

$$\begin{array}{l} \text{Min. Max. Av.} \qquad \text{Min. Max. Av.} \\ L = 0.454-0.912 \text{ (0.685) mm.; } \alpha = 26.4-51.2 \text{ (35.14)} \\ \beta = 4.5 \text{ } -8.8 \text{ (6.4)} \qquad \qquad \gamma = 7.2-25.1 \text{ (13.64)} \end{array}$$

Expressed in Cobb's<sup>5</sup> formula:

ae	sp	bu	nr	po	oepo
.....	(0.8-1.9)	(3.7-8.2)	(7.1-13.7)	(9.1-16.0)	(11.3-22.4)
.....	1.2	5.95	9.36	11.53	14.96
1.1	1.2	1.9	2.1	2.2	2.28
(0.5-1.2)	(0.6-1.9)	(1.3-2.8)	(1.4-3.3)	(1.6-3.0)	(1.5-3.8)
	goae	vu		an	
	(18.4-68.1)	(74.5-83.6)		(85.6-96.0)	
	35.4	79.24		92.08	
	2.5	2.36		1.7	
	(1.7-3.8)	(1.5-3.3)		(1.1-2.5)	

= 0.454-0.912 (av. = 0.685) mm.

B. Average of 36 males.

Expressed in de Man's formula:

$$\begin{array}{l} \text{Min. Max. Av.} \qquad \text{Min. Max. Av.} \\ L = 0.513-0.748 \text{ (0.607) mm.; } \alpha = 31.6-52.0 \text{ (38.2)} \\ \beta = 4.7 \text{ } -7.9 \text{ (6.1)} \qquad \qquad \gamma = 10.1-15.3 \text{ (12.2)} \end{array}$$

Expressed in Cobb's formula:

ae	sp	bu	nr	po	oepo
.....	(0.8-1.5)	(5.8-8.4)	(8.9-13.1)	(10.7-12.4)	(12.9-21.3)
.....	1.2	6.9	10.7	11.7	16.2
0.9	1.1	1.8	2.1	2.1	2.2
(0.6-1.2)	(0.9-1.6)	(1.5-2.6)	(1.8-2.9)	(1.8-2.4)	(1.6-2.9)
	goae	an			
	(28.4-54.3)	(90.2-93.4)			
	38.2	M		91.6	
	2.4	2.6		1.7	
	(1.8-3.1)	(1.9-3.1)		(1.1-2.1)	

= 0.513-0.748 (av. = 0.607) mm.

Specimens from sweet potatoes grown in North Carolina:

A. Average of 26 females:

Expressed in de Man's formula:

$$\begin{array}{l} \text{Min. Max. Av.} \qquad \text{Min. Max. Av.} \\ L = 0.489-1.053 \text{ (0.812) mm.; } \alpha = 31.3-59.5 \text{ (41.6)} \\ \beta = 6.0 \text{ } -10.0 \text{ (7.18)} \qquad \qquad \gamma = 12.5-28.0 \text{ (16.8)} \end{array}$$

$$^4 L = \text{length}; \alpha = \frac{\text{length}}{\text{greatest width}}; \beta = \frac{\text{length}}{\text{length of oesophagus}}; \gamma = \frac{\text{length}}{\text{length of tail}}$$

<sup>5</sup> ae = anterior end; sp = spear; bu = bulb; nr = nerve ring; po = porus of the ventral gland; oepo = posterior end of the oesophagus; goae = anterior end of the ovary, anterior end of the gonad; vu = vulva; an = anus; M = middle of the body length.

Expressed in Cobb's formula:

ac	sp	bu	nr	po	oeo
.....	(0.6-1.2)	(4.5-8.8)	(7.3-9.6)	(10.5-12.3)	(9.9-17.6)
.....	0.8	5.22	8.4	11.2	13.4
0.7	0.8	1.5	1.7	1.9	1.9
(0.4-1.0)	(0.6-1.4)	(1.1-1.8)	(1.4-2.0)	(1.6-2.3)	(1.4-2.4)

goae	vu	an
(28.4-44.4)	(79.5-89.4)	(92.2-96.4)
34.2	83.2	93.5
2.2	1.9	1.3
(1.6-2.8)	(1.2-2.9)	(0.7-1.9)

= 0.489-1.053 (av. = 0.812) mm.

#### SYMPTOMS OF THE DISEASE

Nematode-diseased sweet potatoes exhibit a brown to brownish black layer under the skin. In a more advanced stage of the disease the whole interior shows signs of decay. Often it then contains not only *Anguillulina dipsaci* but also mycelia.

The nemas seem to enter the tubers by preparing openings with their spears. As the disease begins, very few symptoms can be seen on the surface. Here and there sunken portions appear, but there is no true discoloration. Upon cutting the tuber, however, a dark brown layer, 3 to 5 mm. thick, is seen in smaller or larger sections or all around under the cortex. In this layer most of the nemas are found (Fig. 1, B). Later, the brown ring turns almost black and the inner portions of the tuber turn brown. In the final stage of the disease the skin shrinks and crinkles and the whole interior of the tuber becomes black and begins to decay completely.

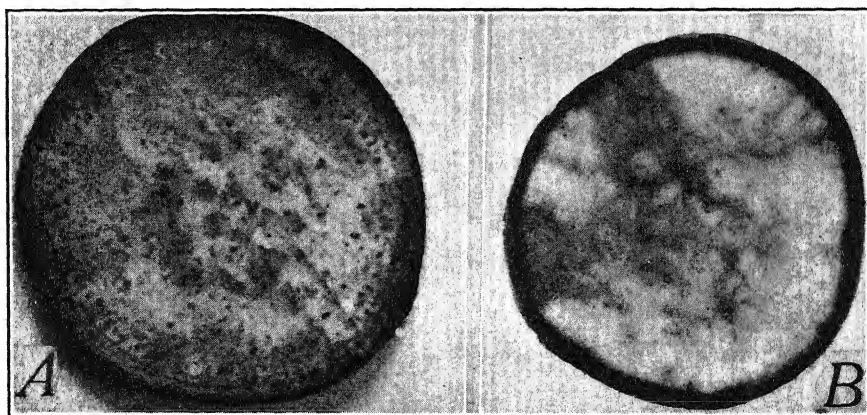


FIG. 1. A. Cross section through a healthy sweet potato; the cambium ring and the vessels are distinct. B. Cross section through a sweet potato infested with *Anguillulina dipsaci*; cambium and vascular bundles have disappeared.

Similar symptoms of a sweet potato disease were described by Elliott (7) in 1918 as occurring in southern Arkansas, suggesting the possibility that the present disease was observed earlier but not properly recognized. The symptoms produced by *Heterodera marioni*, the root-knot nematode (20) on sweet potatoes are quite different. Here the tubers exhibit scab-like abrasions, ring-like lesions, and cracks. The external appearance of such sweet potatoes impresses one that the host reaction to this nema differs considerably from the reaction to *Anguillulina dipsaci*. It was thought that, as in Irish potatoes, the nema also in the present case might attack a tuber by coming down from the green parts of the plant (21), but during these investigations *A. dipsaci* was never observed to attack the green parts of a sweet potato plant, nor did experiments in this connection give satisfactory results. Although the parts of the plant above the ground are in contact with the soil, it was impossible to find the parasite there. It, therefore, is concluded that the tubers are entered directly.

The gradual development of the disease symptoms in the sweet potato and Irish potato is much alike (17, 21). In the latter, as in the former case, the surface of the tuber at first is not changed materially. However, it does become undulated and wrinkled (16) and discoloration takes place after the infection spreads over the whole tuber. Decay of the potato and migration of the parasite follow a similar course. This is confirmed by observations of Ritzema Bos (23) as well as Kühn (16).

#### NUMBER AND DISTRIBUTION OF ANGUILLULINA DIPSAZI IN THE SWEET-POTATO TUBER

It seemed desirable to ascertain approximately how many nemas might be present in a diseased sweet-potato tuber of medium size, how the parasite is distributed through the tuber, and the ratio of the sexes. That plant-parasitic nemas often occur in enormous numbers may be seen from data recorded by Reh (22) for *Anguillulina tritici* (Steinbuch).<sup>6</sup> In 1898-99 Dorph-Petersen reported up to 115,000 infested kernels, in one kilogram of *Holcus lanatus* seed and Rostrup in the same year, found 72,000 infested kernels. Marcinowski (17) mentions an average of 15,000 *A. tritici* individuals in a single wheat gall.

To determine in the present case the total number of nemas in a medium-size sweet potato known to be well infested, a cylinder with a cross-section area of 1 sq. cm. was cut out. This cylinder was sliced very thin, about 1.5 mm. In every slice the nemas were counted and grouped according to sex. Table 1 gives the results of the count for each slice from the outside of the potato inward.

If the size of a potato is assumed to be  $10 \times 3.3 \times 5$  cm., calculated on the

<sup>6</sup> Now *Anguina tritici* (Steinbuch) Filipjev. See citation under footnote 1.

TABLE 1.—*Number of nemas in 1 sq. cm. of a well-infested sweet potato*

Slice	Depth at which the slice occurred	y <sup>a</sup>	♂	(♀)	♀	♀ ♀	Total	m: y	sn	Remarks
	mm.									
1 .....	0- 1.5	1,677	88	44	187	22	2,018	1: 5	34.7	} Nemas occurring in nests
2 .....	1.5- 3.0	127	16	14	23	17	197	1: 25	29.6	
3 .....	3.0- 4.5	29	7	5	7	5	53	1: 1	41.2	
4 .....	4.5- 6.0	9	2	3	4	1	19	1: 1	25	
5 .....	6.0- 7.5	.....	5	2	5	5	17	.....	41.5	
6 .....	7.5- 9.0	4	5	3	5	2	19	1: 0	50	
7 .....	9.0-10.5	.....	8	4	5	5	22	.....	57	
8 .....	10.5-12.0	15	2	1	3	1	22	1: 2	40	
9 .....	12.0-13.5	9	.....	1	2	1	13	1: 2	.....	
10 .....	13.5-15.0	3	.....	2	4	2	11	3: 1	.....	
11 .....	15.0-16.5	7	.....	1	1	3	12	1: 1.5	.....	
Total .....		1,880	133	80	246	64	2,403	<sup>b</sup> 1: 3.6	<sup>b</sup> 39.9	

<sup>a</sup> y = young specimens; (♀) = immature female; ♀ = mature female; ♀ ♀ = mature female with eggs; m: y = mature specimens: young specimens; sn = sex number.

<sup>b</sup> Average.

findings of table 1, the infestation numbers of the various layers and of the whole potato, together with their proportionate distribution expressed in per cent, are set forth in table 2.

TABLE 2.—*Number of nemas in the various layers of an infested sweet potato with proportionate distribution as expressed by percentages*

Layer	♀ ♀	♀	♂	y	Total	♀ ♀	♀	♂	y
	number	number	number	number	number	per cent	per cent	per cent	per cent
1 .....	4,950	51,977	19,801	377,342	454,070	1.1	11.4	4.8	83.0
2 .....	2,646	5,758	2,490	19,764	30,658	8.6	18.8	7.5	64.3
3 .....	683	1,638	956	3,960	7,237	9.5	22.6	13.2	54.7
4 .....	119	830	237	1,067	2,253	5.3	36.8	10.5	47.4
5 .....	508	711	508	.....	1,727	29.4	41.2	29.4	.....
6 .....	172	686	429	353	1,640	10.5	41.8	26.1	21.5
7 .....	355	639	568	.....	1,562	22.7	40.8	36.3	.....
8 .....	57	229	115	860	1,261	4.5	18.2	9.1	68.2
9 .....	48	134	.....	403	585	8.2	22.8	.....	69.0
10 .....	66	199	.....	100	365	18.2	54.5	.....	27.4
11 .....	68	45	.....	160	273	24.9	16.5	.....	58.6
Total	9,672	62,846	25,104	404,009	501,631	1.9	12.5	5.1	80.5

Figure 2 shows the proportionate distribution of the 4 groups; females with eggs, females without eggs, males, and young specimens. Although 501,631 specimens were counted, it must be emphasized that the final number is too small rather than too large. It showed that the distribution is not uniform throughout the tuber; near the center, especially in the fifth

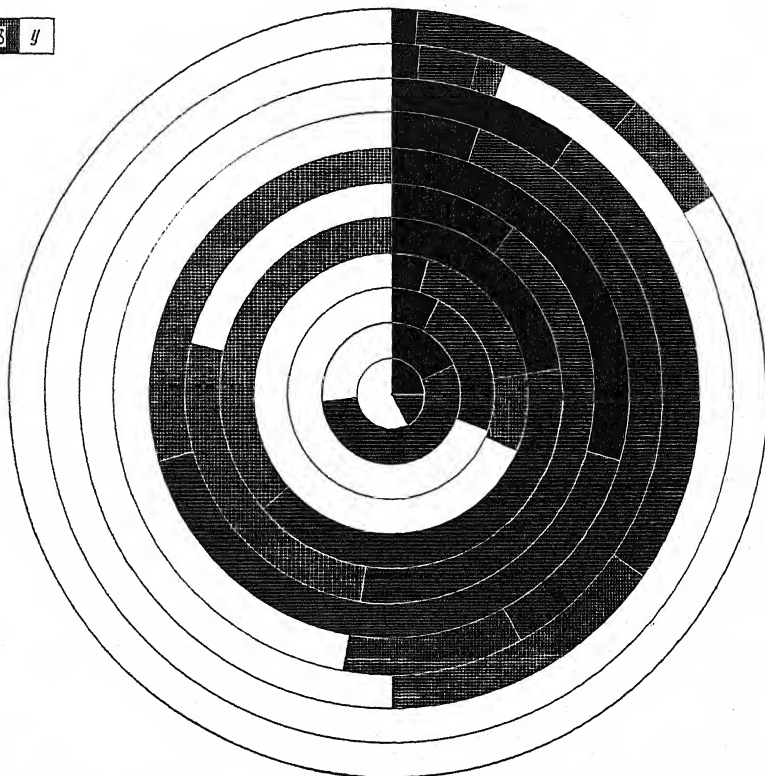


FIG. 2. Diagram showing the distribution of *Anguillulina dipsaci* within the several layers of an infested sweet potato. Females with eggs, ♀ (cross-hatched areas); females without eggs, ♀; males, ♂; and young specimens, y.

to eighth layers, the nemas occur in nests. An explanation of this fact cannot be given; the internal structure of the tuber is considered a possible reason. Toward the center the males gradually disappear. It is significant that the females are the first to penetrate the center regions of the tuber and, hence, seem more active than the males. The decrease in numbers from the outside toward the center is rapid after the first layer. While the latter contained 454,070 nemas, the second and third layers had only 30,658 and 7,237, respectively. This was probably caused by the distribution of suitable food material in the tuber, it being richer just under the skin.

Table 3 shows the comparative distribution of the two sexes and the larvae through the tuber. A predominance of the larvae is evident and proves that a very active propagation takes place within the tuber.

#### TRANSFER EXPERIMENTS

It is well known that the transfer of the present parasite from one host plant to another is not easy. After living for a few generations on the same

TABLE 3.—Comparative distribution of nemas through a well-infested sweet-potato tuber

Slice	♀	♂	γ
	number	number	number
1 .....	100	35	592
2 .....	100	30	235
3 .....	100	41	170
4 .....	100	25	127
5 .....	100	42	.....
6 .....	100	50	41
7 .....	100	57	.....
8 .....	100	40	300
9 .....	100	.....	221
10 .....	100	.....	38
11 .....	100	.....	141

host, the parasite seems to adapt itself especially to this particular host. Both Ritzema Bos (23) and Marcinowski (17) concluded that the longer a nema lives in a plant the less adaptable it becomes to a new host.

The present experiments were made with the following questions in view:

1. Does *Anguillulina dipsaci* attack only the tuber or does it also attack the green parts of the sweet-potato plant?



FIG. 3. Methods of inoculation and isolation of the living sweet-potato plant: *a*, leaf and *b*, bud.

2. Is a transfer from the sweet potato to the Irish potato possible?

3. Is an infection of healthy sweet-potato tubers possible if they are brought in contact with structures of other plants infested with this nematode, such as narcissus bulbs or tubers of Irish potatoes?

Unfortunately, because of lack of time, the experiments could not be reversed, which would have been desirable.

#### Transfer Experiments from Sweet Potato to Sweet Potato Plant

A series of six experiments was conducted to attempt the inoculation of the green parts of the sweet-potato-plant.

1. A lesion was made on the stem of a sweet-potato plant and *Anguillulina dipsaci* inoculated. After 2 days the stem dried up and withered. A repetition of the experiment gave the same result. The death of the stem was apparently due to the injury.

2. A drop of water was placed in the axil of a leaf and specimens of *Anguillulina dipsaci* were added. Five days later the leaf was decayed and the parasite not established. Some of the specimens were recovered, but they were dead. A repetition gave the same result.

3. The surface of a leaf was injured, moistened with water, inoculated with the parasite, and placed in a moist chamber (Fig. 3, a). The same experiment was repeated. In both cases after 2 weeks the leaf dried up. Some of the nemas were still living, though the majority were dead. An infection did not take place.

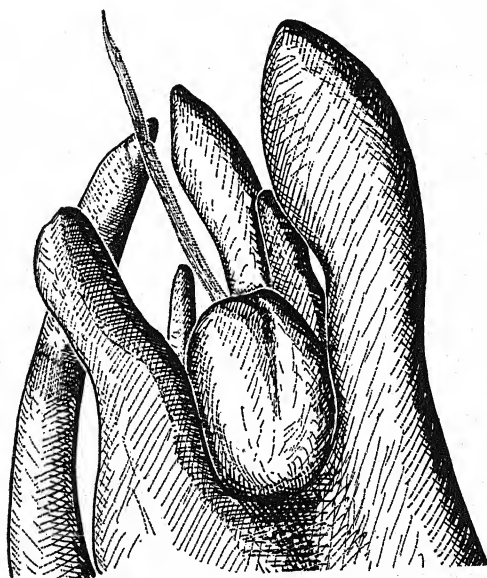


FIG. 4. *Anguillulina dipsaci* penetrating the young bud of a sweet-potato plant.

4. The experiment in no. 3 was repeated except that the underside of the leaf was injured. After 2 weeks all nemas were dead, but the leaf was still living. Only a scar could be seen.

5. A piece of a diseased sweet potato was tied to a green stem with thread. Four days later the stem turned yellow and after 6 days it was dead. The piece used for infection still contained numerous *Anguillulinae*. The decay of the stem was due to the thread, which cut into the tissues.

6. Thirty specimens of the nema were put into a drop of water, the slide placed in a moist chamber (Fig. 3, *b*) and brought in contact with a young bud. After 2 days it was observed that 22 nematodes were still in the

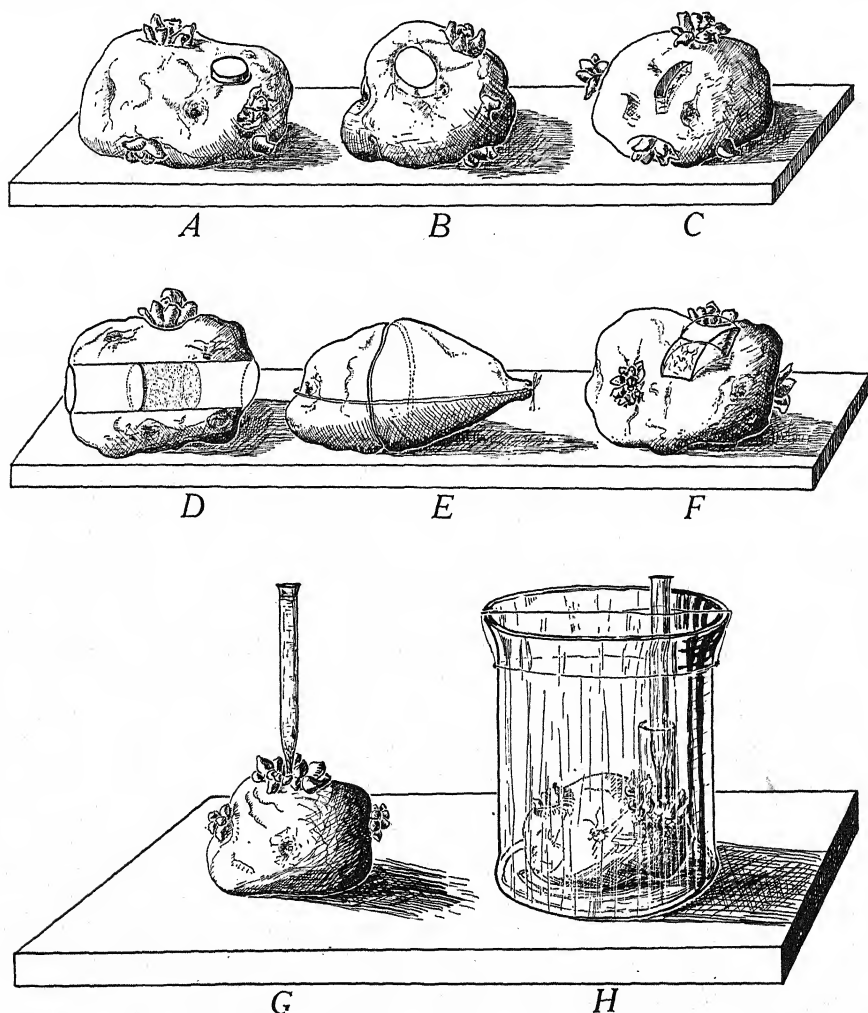


FIG. 5. Various technical methods used in the transference experiments of *Anguillulina dipsaci*.



water, 4 had crawled up along the young plant, and 1 had bored into the tissues of the bud (Fig. 4), the latter exhibiting a brown discoloration. The experiment was repeated, and after 2 days eighteen nematodes were still in the water, 6 had crawled up along the young plant, and the rest could not be found. An infection of the bud did not take place.

A second series of 6 experiments was started to attempt the transfer of the nematode from tuber to tuber. Each experiment was performed in duplicate. To approximate conditions in the storage houses, the temperature was held between 55° and 58° F.

1. A piece of the skin of a diseased sweet potato was fixed to a healthy tuber with the aid of a fine needle (Fig. 5, A). The transfer was successful in one case but not in the other.

2. A circular piece was cut from the surface of a healthy tuber and a similar piece of a diseased one inserted (Fig. 5, B), but for some reason the transfer was unsuccessful.

3. A cylindrical piece about 1 cm. in diameter was taken from a healthy potato and cut into 3 parts. A piece, equal in size to the middle part, was taken from a diseased tuber and inserted in the place of the middle third of the cylinder (Fig. 5, D). This new tripartite cylinder was replaced in the healthy tuber; in both tubers so treated the disease transferred.

4. The halves of a healthy and a diseased potato were bound together (Fig. 5, E) but fungus growth developed and ended this experiment. A healthy and a diseased tuber were placed in contact, the places of contact having been previously moistened. The disease transferred.

5. A piece of skin was carefully removed from a healthy tuber. Twenty-four living and mature *Anguillulinae* were placed in the wound and the skin was replaced (Fig. 5, F). Both tubers became soft; mycelium had developed and apparently killed the nemas.

#### Transfer Experiments from Sweet Potato to Irish Potato Tubers

A similar series of experiments (Fig. 5, G and H) was made to see whether a transfer of the disease from the sweet potato to the Irish potato could be accomplished. A surface scar was made and 36 *Anguillulina dipsaci* from a sweet potato were inserted. Also an open glass tube containing a number of *Anguillulinae* in water was inserted into a tuber; strangely the nemas did not enter the tissues of the latter but remained in the tube. Then a tuber was placed in water containing numbers of *A. dipsaci*. The results were always negative.

#### Transfer Experiments with *A. dipsaci* from Other Hosts to Sweet Potato

*From Irish Potato.* 1. A sweet potato was cut in 2 parts and a piece of a diseased Irish potato was placed on one part. No nemas were found to have transferred.

2. A piece of diseased Irish potato was substituted for the middle section of an excised cylinder. In this case many living nemas transferred and were observed in the center portions of the sweet potato.

3. Twenty-four living *Anguillulina dipsaci* from an Irish potato were transferred to a sweet potato (Fig. 5, F). A greenish white mold, however, developed and covered the injured space; part of this region became brown and hard. No transferred nemas could be found.

*From Narcissus.* 1. A similar series of transfer experiments was made with *Anguillulina dipsaci* specimens from narcissus bulbs. In only one case was a positive transfer observed.

### Summary of the Results of Transfer Experiments

If these preliminary experiments permit any conclusions, they are the following: The green parts of a sweet-potato plant are neither attacked, nor invaded except under special conditions. The transfer from sweet-potato tuber to sweet-potato tuber takes place best by contact of the tubers. Transfers from sweet-potato tubers to Irish-potato tubers were unsuccessful. The reverse, however, was observed in a single case. Some specimens also entered a cut sweet potato from an inserted piece of narcissus bulb, but since none of these experiments was of long enough duration, it is not known if in all cases the parasite would have established itself in the new host.

### A STUDY OF CONTROL MEASURES

The use of various nematocides and other control measures has been tried for control of *Anguillulina dipsaci*. Kühn (16) suggested dipping the potato tubers in hot water. Marcinowski (17) experimented with carbon bisulphide, caustic-lime, and a mixture of hot water with 0.66 per cent sulphuric acid. The latter solution, allowed to act for 24 hours on infested onions killed the nemas. Cobb (3) found hot water fatal to *Tylenchulus semipenetrans* Cobb on citrus roots. He found that the vitality of the parasite *Anguillulina pratensis* (de Man) (= *Tylenchus penetrans* Cobb) in scabby potatoes was reduced by treatment with mercuric chloride (4). Several chemicals were tested by Ritzema Bos (23) and some were found to be lethal to the nemas if applied for a certain length of time.

A nematocide must be satisfactory not only as to lethal effect on the parasite but also as to price, facility of application, adaptability to farm practice, and freedom from danger to man. In the search for such a chemical, the preparations already in use by farmers for various purposes were tried first.<sup>7</sup> A widely used disinfectant is Semesan Bel, a mixture of 12 per cent hydroxymercurinitrophenol and 2 per cent hydroxy-

<sup>7</sup> The selection of the different disinfectants was not arbitrary. Chemists were consulted, and we are much indebted to Dr. H. A. Peter of New York, and Prof. Dr. H. Kreis, in Basel, Switzerland, for their advice.

mercurichlorophenol. A few farmers go their own way and try all sorts of substances. Such cases will be mentioned in their proper place. Altogether, 24 different experiments were tried. Along with a normal sweet potato a diseased tuber was planted in each test pot used to test a disinfectant. In addition 2 controls were made, 1 with a healthy sweet potato only, the other with a healthy and a diseased tuber. The experiments were started on March 26, 1931. Sterilized soil was used to which was added a mixture of 5 g. potash ( $K_2CO_3$ ) and potassium chloride (KCl) in the proportion of 1:1 as fertilizer.

It may be noted that a control tuber placed with a diseased tuber became infested within 1 month. The parasite was generally absent from decayed tubers. However, it appears that in these instances the treatment killed the tubers, as well as the parasites. Several treatments with various disinfectants were conducted to ascertain the effectiveness of each as a control agent. The results are presented in table 4 and subsequent text.

#### EFFECT OF VARIOUS DISINFECTANTS

*Copper Sulphate ( $CuSO_4$ ) and Mixtures of It.* After treatment in a  $12\frac{1}{2}$  per cent aqueous solution of  $CuSO_4$ , applied for 30 minutes, the potato slowly sprouted; it did not sprout at all in a solution of  $CuSO_4$  and  $NaNO_3$  (10 per cent), but seemed in good condition.

*Phenol ( $C_6H_5.OH$ ) and Mixtures of It.* Phenol is well known as a powerful antiseptic. It is used as a disinfectant in storage houses. In the present instance it killed the tuber. Mixed with lime, however, it so affected one plant that it was the first to sprout and the resulting plant proved to be one of the best-developed at the end of the experiments. It was 8 inches high on April 30. Phenol seems to be one of the best disinfectants favorable to the plant and destructive to the parasite. Additional experiments are desired. Application is easy and costs are not prohibitive.

*Formaldehyde ( $H.CHO$ ) and Mixtures of It.* The physiological effect of a solution of 4 per cent  $H.CHO$  on animals seems different than on plants. Nemas are killed quickly, while a sweet-potato tuber sprouts, although a little delayed, in spite of a 15-minute exposure. A mixture of  $H.CHO$  and  $NaNO_3$  gave the impression that  $H.CHO$ , compared with the mixture of  $CuSO_4$  and  $NaNO_3$ , reduces the dangerous effect of the nitrate. The sweet potato grows better than after disinfection with  $H.CHO$  alone. Perhaps in the present case the tuber utilizes the  $NO_3$  group, which for some reason it is unable to use in the  $CuSO_4$  and  $NaNO_3$  combination. The action of a mixture of the 4 chemicals, permanganate, sodium carbonate, potash, and formaldehyde, was never clear to me. It is apparent that the carbonates are not only unnecessary but they also stop the reaction of the permanganates and thus allow the evaporation of  $H.CHO$ . The effect of the mixture is only a

TABLE 4.—Results of treatments with various disinfectants employed as nematocides

Treat- ment no.	Disinfectant	Length of treatment	Results April 24	Results April 30	Remarks
1 .....	Control	.....	.....	coming up .....	without diseased tuber
2 .....	Control	.....	.....	coming up .....	with diseased tuber; infected with <i>Anguillulina dipsaci</i>
3 .....	Copper sulphate (12½ per cent)	30 minutes	sprouting decayed	coming up .....	without nematodes
4 .....	Phenol (2 per cent)	do do	sprouting decayed	coming up well	
5 .....	Potassium permanganate (5 per cent), sodium carbonate (10 per cent), potash (10 per cent), and formaldehyde (4 per cent)	do do	sprouting	no sprouting	tuber still in good condition without any signs of devel- opment
6 .....	Copper sulphate (12½ per cent) and sodium nitrate (10 per cent)	60 do	no sprouting	do	3 tablespoons lime and 3 table- spoons Na <sub>2</sub> CO <sub>3</sub> and 2 pints H <sub>2</sub> O
7 .....	Lime and sodium carbonate	5 do	do	coming up very quickly	the tuber sprouted April 18
8 .....	Lime and phenol (1 per cent)	15 do	sprouting	no sprouting	seurf without nematodes
9 .....	Mercuric chloride (3 per cent)	60 do	decayed	coming up .....	
10 .....	Sodium nitrate (10 per cent)	do do	sprouting	coming up .....	
11 .....	Formaldehyde (4 per cent)	15 do	sprouting	.....	
12 .....	Potassium carbonate (10 per cent) and potassium chloride (10 per cent)	60 do	decayed	.....	without nematodes; propor- tion 1:1
13 .....	Formaldehyde (4 per cent) and sodium nitrate (10 per cent)	15 do	sprouting	coming up well	

TABLE 4.—Results of treatments with various disinfectants employed as nematocides—(Continued)

Treat- ment no.	Disinfectant	Length of treatment	Results April 24	Results April 30	Remarks
14 .....	Toluol	24 hours	decayed	.....	exposed to vapors; without nematodes
15 .....	Carbon disulphide	do	do	.....	do
16 .....	Toluol and carbon disulphide	do	do	.....	do
17 .....	Carbon tetrachloride	do	do	.....	do
18 .....	Toluol and carbon disulphide and carbon tetrachloride	do	do	.....	exposed to vapors; without nematodes (proportion 1: 9:10)
19 .....	do	do	do	.....	exposed to vapors; without nematodes (proportion 6: 3:1)
20 .....	Solution of caustic potash and salt (10 per cent)	2 do	partly decayed	completely decayed	proportion 1:1; without nem- atodes
21 .....	Toluol and carbon tetrachloride	24 hours	one end decayed and cut off	sprouting	proportion 7:3; exposed to vapors
22 .....	Toluol and carbon tetrachloride and carbon disulphide	do	decayed	.....	proportion 75:5:20; exposed to vapors; without nema- todes
23 .....	Semesan Bel (6.6 per cent)	1 minute	sprouting	coming up	
24 .....	do	10 do	do	do	

disinfection through  $\text{H.CHO}$ ; neither permanganate nor the carbonates are necessary (similar to treatment no. 13 in Table 4).

*Lime and Sodium Carbonate* ( $\text{Na}_2\text{CO}_3$ ). The effect is the same as if  $\text{Na}_2\text{CO}_3$  alone were used. No sprouting took place; the tuber, however, was still good.

*Potassium and Sodium Combination.* The destructive effect of  $\text{NaNO}_3$  is revealed in treatment 10. The tuber was unable to make use of the  $\text{NO}_3$  group. The same effect resulted from applying the fertilizer  $\text{K}_2\text{CO}_3$  and  $\text{KCl}$  as a disinfectant. It stimulates growth, but kills the tuber if brought in direct contact. Applying the fact that  $\text{NaCl}$  kills freshwater nematodes (14), an experiment with this substance combined with  $\text{KOH}$  (No. 20) was made. But solutions of different strengths only brought about disturbances in sprouting, and the tubers decayed.

*Exposure to Vapors.* The usefulness and practicability of fumigation methods for sweet-potato disinfection was considered with much skepticism as to its effect on an *Anguillulina dipsaci* infestation, although farmers fumigate for various purposes. The following preparations were tried:

*Toluol* ( $\text{C}_6\text{H}_5\text{CH}_3$ ), which has a decided antiseptic character.

*Carbon disulphide* ( $\text{CS}_2$ ), which is a well-known remedy against bots (*Gastrophilus*) in horses and is also used to control various nemic pests in the soil.

*Carbon tetrachloride* ( $\text{CCl}_4$ ), which is used to control worm parasites of animals. The reason for the application of this latter chemical is its nonexplosive character compared with  $\text{CS}_2$ .

In each case the tubers were fumigated for 24 hours and then planted, but they all decayed. Similar results were observed with mixtures of fumigants except in experiment 21 where the tuber, although slightly decayed at one end, began to sprout after the healthy portion was planted. An explanation is rather difficult. It may be that an interaction of both fumigants changes them so that each loses its destructive effect upon the tuber.

*Semesan Bel.* This disinfectant gave positive results in both experiments, though the growth of the tuber was very much reduced after remaining 10 minutes in a 6.6 per cent solution. The tuber of experiment 24 was just beginning to grow while that of experiment 23 developed the first leaves. A protracted treatment of tubers with Semesan Bel cannot be recommended.

These experiments show that the use of known fumigants is not advisable for sweet potatoes; furthermore, some are too expensive and dangerous. For the farmer only a disinfectant that is easily applied and devoid of danger should be considered. Lime and phenol offer these qualities. The disinfectant acts at once as a nematocide, a stimulant, and a fertilizer.

Various other chemicals should also be tested for their possible application as disinfectants. Chlorine should be considered first. It is very soluble

in water and enters the tuber through the respiratory cells. The parasite is certain to be killed. Another suggestion would be to apply different mixtures of sodium hydroxide and phenol. The germicidal efficiency of these was studied by Schaffer and Tilley (25, 27), who concluded that these mixtures were effective against the most diverse germs. Consequently, there is reason to suppose that they might be effective against nemas. Also paradichlorobenzene ( $C_6H_4Cl_2$ ) could be especially considered for field application. The crystallized commercial form of this chemical has been applied on peach farms against dangerous insects. In soil it possibly remains active for 2 weeks. There is no denying that control for all soil parasites is very difficult. If unsuccessful, there remains the possibility of breeding disease-resistant varieties.

#### DISCUSSION OF FIELD, SEED, AND STORAGE CONDITIONS AS RELATED TO THE PRESENT PROBLEM

##### Field Conditions

During field investigations the difference between disease conditions in a well-cared-for planting and a neglected one was very plain. Quanjer (21) was right in stating that an energetic eradication of the weeds is the first preventive against plant diseases, including those caused by nemas. Many weeds are not only hosts of *Anguillulina dipsaci* but also of other parasitic and semiparasitic nemas; and the more they develop the more dangerous they become for crop plants. Inquiries concerning the number of successive years during which sweet potatoes were grown in a field showed that near Swedesboro, New Jersey, where *A. dipsaci* developed in the spring of 1930, sweet potatoes often are grown too many years in succession. This is especially favorable for the development of nemie diseases.

##### Seed Conditions

It is evident that cultural control of nemie diseases is without avail if infested seed potatoes are planted. All authors who have discussed the nemie diseases of plants emphasize the fact that a very careful selection of seedling plants is one of the main control measures (1, 5, 11, 20, 21, 29).

##### Storage Conditions

A temperature of 70° to 80° F. is about optimal for *Anguillulina dipsaci*. Unfortunately, the storage temperature for sweet potatoes during the winter is not far below the optimum, and leaves the nemas more or less fully active in the storage houses. This may account for the fact that the malady here considered is mainly a storage, rather than a field disease, at least as far as yet known. It is of some interest to compare with this condition the temperature of storage houses in various localities of the infested region. Harter and Weimer (11) suggest that sweet potatoes should be kept as near 55°

as possible after remaining 2 weeks at a temperature of 80° to 85°. DeBaun (5) proposes 80° in the first period and 50° in the second. The temperatures in °F. at which sweet potatoes were kept in storage in various sections visited by the writer were as follows:

Snow Hill, Maryland .....	46 to 48
Mullica Hill, New Jersey .....	over 50
Vineland Section, New Jersey:	
Place A, first period .....	75
“ “ second “ .....	50 to 55
Place B, first “ .....	65 to 70
“ “ second “ .....	55 to 60
Place C, “ “ .....	60 to 65
Place D, “ “ .....	45
Ewan, New Jersey, second period .....	45 to 50
Swedesboro, New Jersey, second period .....	about 60

*Disinfectants used in Storage Houses.* At one place visited, formaldehyde was applied. At another lime and carbolic acid. More often, however, the latter were employed in conjunction with Semesan Bel. Also, copper sulphate, or a mixture of lime and soap, together with toluol, carbon disulphide, and carbon tetrachloride, were applied. At other places no disinfection was undertaken. During the average duration of a treatment the disinfectant should penetrate every crevice of floor and wall. Eggs and nemas in dormancy are very resistant.

#### BIOLOGICAL OBSERVATIONS

Not only the number of nemas parasitizing a plant, but, also, climatic and other conditions under which this plant grows are factors contributing to the severity of disease thus produced. The developmental stage of a plant, according to the writer's observation, is very important because of the host's susceptibility to an attack by *Anguillulina dipsaci*. During the early period of development of aboveground parts and of the first roots, the plant is much more susceptible than later when it is more fully grown. The early growth is much more rapid. It seems less able during this period to protect itself sufficiently against the attacks of the parasites. This is why an entire seedbed may decay within a short time, as was observed in Monroeville, New Jersey.

It has been emphasized that the parasite is of cosmopolitan distribution. The number of plant species attacked by *Anguillulina dipsaci* seems on the increase because of the adaptability of the parasite. At present, as already mentioned, some 250 various host plants are known. Kühn (15), then later Hodson (12), Quanjer (21), de Bruyn Ouboter (2), and other authors showed that all stages of *A. dipsaci* can be found in a host. The nema does not propagate outside of its host; it is a true parasite. All stages of development were found fully active in the tubers during the winter months (12). Although *A. dipsaci* is a true parasite, it is able to exist free in the soil (1, 16,



17); otherwise, it could not transfer from one host to another (8, 22, 23). There is an active migration of the parasite into the new host, but there is no obligate free-living stage. Parasitism is the normal status of *A. dipsaci*, and only adverse conditions force it to leave the old host and to stay free in the soil until a new one is available. As soon as a host plant decays, wholesale migration into the soil begins, chiefly by the preadult larvae, which are very resistant to adverse conditions and may ultimately become dormant.

Migration of *Anguillulina* may be dependent also upon such climatic conditions as frost and drought (17). Drought, under certain conditions, may account for the invasion of tuberous plants such as Irish potato and sweet potato, which are excellent water reservoirs and, for that reason, sometimes the only place for the parasite to go, unless it becomes dormant.

Baunacke (1) showed that the distance traveled by *Heterodera schachtii* Schmidt increases with the approach of optimal temperature. Increasing temperature in the field, however, has the effect of hastening evaporation of water, which then often checks motility. The attack on the host is to some degree a function of climatic conditions. Under adverse conditions a plant is less able to offer resistance. If no favorable hosts are available, it generally is agreed that *Anguillulina dipsaci* lapses into a state of suspended animation. Goodey (10) showed by experiments with onions and narcissus that *A. dipsaci* is able to remain 2 years in a latent stage and then return to full activity. Still other authors mention a much longer possible dormancy. This is a characteristic that pertains to a number of species of nemas.

Its remarkable viability contributes much to the fact that *Anguillulina dipsaci*, once in a field, cannot readily be stamped out. A migration from the host takes place as soon as mites and fungi appear as attendant phenomena of the decomposition of the plant. Quanjer (21) saw this in the Irish potato. It also was observed in sweet potatoes. First, the mature specimens escape from the host, then the young nemas; the eggs, which remain in the plant, dry out. Almost without exception completely decayed sweet-potato tubers harbored only young specimens in a lethargic state, which soon became fully active when put into water.

#### OBSERVATIONS ON VARIABILITY IN *ANGUILLULINA DIPSACI*

Parasitic nemas are more variable than free-living ones. According to Micoletzky (18), the average deviation of the females is greater than that of the males. The present biometrical investigations were made on 100 females and 36 males out of a single tuber of a sweet potato that came from New Jersey. As it was difficult to obtain 100 females with eggs, measurements include also other fully developed specimens. The size varies between 0.454 and 0.912 (0.685) mm. Hence, it follows that the degree of variation is -33.7 per cent and +33.1 per cent for the New Jersey specimens. For

North Carolina specimens it was found to be  $-39.8$  per cent and  $+29.8$  per cent. This degree of variation corresponds approximately with the one-third variation noted by Micoletzky.

The details of methods of calculations of these variations cannot be given here. The most important results are:

The length of the tail, the parts of the oesophagus, and the width of the body exhibit considerable variation.

The female exhibits greater variation than the male.

In the female three types of anterior ends are present; besides a normal type there are specimens having an extremely long or an extremely short



FIG. 6. Series of posterior ends of females of *Anguillulina dipsaci* showing the variations in the vulva-anus distance and the length of the tail. A. Normal proportion of the vulva-anus distance to the length of the tail. B. and C. Specimens showing increasing proportion in the length of the tail. D. and E. Specimens showing the length of the tail greater than the vulva-anus distance. F. Specimen showing a proportionately very short tail.

oesophagus. However, it would be wrong to attempt to differentiate species on the basis of the length of the oesophagus.

The average proportion of the distance, vulva-anus: length of tail = 1.85:1. Figure 6A gives the normal proportion. Figure 6B and C demonstrate a proportionate increasing length of the tail to a point where its length surpasses the distance vulva-anus (Fig. 6, D and E). On the other hand the distance vulva-anus may become so great that the tail compared with it seems relatively short (Fig. 6, F). The question arises, on the basis of these facts, as to whether the following groups could be distinguished:

1. *Anguillulina dipsaci forma media*, including specimens with the proportion vulva-anus: length of tail = 1.1–2.9:1.

2. *Anguillulina dipsaci f. brevicaudata*, including those with the proportion vulva-anus: length of tail = 3 or more:1.

3. *Anguillulina dipsaci f. longicaudata*, including those with the proportion vulva-anus: length of tail = 1:1 or more.

#### A COMPARISON BETWEEN ANGUILLULINA DIPSACI FROM SWEET POTATOES AND FROM OTHER HOSTS

It was shown that at least some of my transfer experiments were positive. The question, therefore, arises as to the polyphagous or partly monophagous character of the present race. A comparison of measurements of my specimens with those of other authors (see Table 5) shows very distinctly that variations were observed in all instances. The 3 suggested *formae* again may be recognized, but the population of the sweet potato seems to consist of specimens of the smallest size. *Vicia faba* apparently harbors besides the *forma media* also *f. longicaudata*; the same seems true for the population which Micoletzky (18) found in grass roots. However, *Amsinckia intermedia* (26) seems to harbor almost exclusively the short-tail form.

A high degree of variability, therefore, is to be found in the population of not only sweet potatoes but of other hosts, also. The latest studies of this kind on *Anguillulina dipsaci* are those by de Bruyn Ouboter (2). This author concludes that narcissus and hyacinth harbor different races, which she calls *Tylenchus devastatrix narcissus* and *T. devastatrix hyacinthus*.

It is questionable whether this conclusion is correct. That the individual is not a full realization of the species is an accepted fact. Johannsen (13) says that the species is a stream of forms that run parallel. If one accepts the interpretation of de Bruyn Ouboter, every host form should have its own name. This would lead to inadequacies, especially in consideration of the ever-increasing number of hosts attacked by *Anguillulina dipsaci*. The experiments here recorded support the possibility of a transfer of the parasite from one host to another, e.g., from narcissus to sweet potato. Referring also to results of other authors, it may be concluded that even the narcissus strain

TABLE 5.—Measurements<sup>a</sup> of specimens of *Anguillulina dipsaci* from various hosts

Host or host organs	Author	Female				
		Length	$\alpha$	$\beta$	$\gamma$	Vulva position Per cent
<i>Dipsacus fullonum</i> .....	Kühn, 1857	0.94-1.144	35.8	7-11	5-18	80.0-81.1
<i>Vicia faba</i> .....	Debray and Maupas, 1896	1.258-2.216	35-41		13.7-15.9	74-80
<i>Dianthus barbatus</i> .....	Steiner [unpublished]	1.1-1.5	59-71.5		20-25	83-91
<i>Amsinckia intermedia</i> .....	Steiner and Scott, 1935	1.07-1.42	25.7-29.4	6.7-7.7	5.6-11.7	72-82
Roots of grass .....	Micoletzky, 1922	0.445-1.05	22-46	5.3-9.3	12-18	80.7-81
<i>Secale cereale</i> .....	Ritzema Bos, 1892 (24)	1.03-1.44	33-50	7-7.15	15.6-15.8	
<i>Narcissus</i> .....	de Bruyn Ouboter, 1930	1.424-1.447	37.8-39		12-17.5	
<i>Allium cepa</i> .....	Ritzema Bos, 1892	1.43-1.73	37-47		11-18	
<i>Hyacinthus</i> .....	Ritzema Bos, 1892	1.07-1.67	31-49	6.35-6.5	15.3-15.6	79.5-79.8
<i>Solanum tuberosum</i> .....	de Bruyn Ouboter, 1930	1.251-1.280	37.4-38.1		15-17	
	Ritzema Bos, 1892	1.320-1.65	40.5-43		13-15	
	Wollenweber, 1923 (30)	0.80-1.29	29-41		10-16	
	Quanjér, 1927	1.056-1.408	32-52	7.2-8.2	17.6-19.8	84.1-84.9
	Kreis	1.014-1.206	28.9-31.4	4.5-8.8	7.2-25.1	75.6-83.6
	Kreis	0.454-0.982	26.4-51.2	6-10	12.5-28.0	79.5-89.4
	Kreis	0.489-1.053	31.3-59.5			
Male						
<i>Dipsacus fullonum</i> .....	Kühn, 1857	1.036-1.269	41	6.4-9	15	
<i>Vicia faba</i> .....	Debray and Maupas, 1896	1.015-2.016	36-61		11-18	
<i>Dianthus barbatus</i> .....	Steiner [unpublished]	1.2-1.4	55.5-59		14.1-15.9	
<i>Amsinckia intermedia</i> .....	Steiner and Scott, 1935	1.04-1.30	25.7-32.3	5.2-7.6	15.4-17.2	
Roots of grass .....	Micoletzky, 1922	0.46-0.98	33-56	4.4-6.4	8.4-11.2	
<i>Secale cereale</i> .....	Ritzema Bos, 1892	1.01-1.47	34-51		11.5-17	
<i>Allium cepa</i> .....	Ritzema Bos, 1892	1.43-1.57	41-50	6.5-6.6	14-16.5	
<i>Narcissus</i> .....	de Bruyn Ouboter, 1930	1.312-1.334	44.6-45.4		15.4-15.7	
<i>Hyacinthus</i> .....	Ritzema Bos, 1892	1.18-1.70	37-51		12-17	
<i>Solanum tuberosum</i> .....	de Bruyn Ouboter, 1930	1.210-1.238	43.0-43.9	6.25-6.4	15.6-15.9	
	Ritzema Bos, 1892	1.35-1.58	43		15-17	
	Kreis	0.723-0.872	33.4-34.7	6.3-6.6	16.7-17.2	
	Kreis	0.513-0.748	31.6-52.0	4.7-7.9	10.1-15.3	

<sup>a</sup> As to  $\alpha$ ,  $\beta$ ,  $\gamma$ , see p. 669.

is not absolutely specialized on narcissus. Very specialized races have been observed but also highly polyphagous ones (21).

Even for the same host pronounced differences were noted in populations from different geographical localities, as Debray and Maupas (6) state that in *Vicia faba* the parasite is about  $1\frac{1}{2}$  times larger in Algeria than in France.

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## FROSTY MILDEW OF PEACH<sup>1</sup>

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A disease of peach foliage, commonly known as "frosty mildew," is more or less familiar to peach growers throughout the world; but usually the damage to the trees is slight and the disease is of minor importance. It is caused by a fungus, the conidial stage of which is best known as *Cercospora persica* Sacc. The writers have had this organism under observation for several years, special attention having been devoted to its morphology and cycle of development. The most important fact gained from these observations is that the pathogen possesses an ascigerous stage, hitherto unknown. The publication of a report on these studies has been delayed pending a complete understanding of the developmental morphology of this ascigerous stage. Since the clarification of certain essential details has been found to be both difficult of accomplishment and very time-consuming, and therefore would unduly delay the presentation of our observations, it appears desirable now to submit, an incomplete report. The following account, therefore, embodies a portion of our studies dealing with the primary causal agent of the frosty mildew disease.

### APPEARANCE OF FROSTY MILDEW AND ITS DISTRIBUTION

This disease, as its name indicates, is characterized by the presence of white, powdery patches on the leaves. These patches occupy the lower leaf surfaces and simulate the appearance of the powdery mildews (Fig. 1). The disease first makes its appearance in June and may be found at any time thereafter until the leaves have been shed. If affected leaves are viewed from the upper side, usually little or no discoloration is apparent, even when the pathogen has reached the fruiting stage. Eventually, indefinite yellowish areas are discernible, however, and during late fall these infected areas may

<sup>1</sup> We are grateful to Dr. D. H. Linder, for examining for us exsiccati in the Farlow Herbarium, and to Dr. H. A. Edson for distributional data on this organism from records in the Division of Mycology and Disease Survey, U. S. Department of Agriculture.

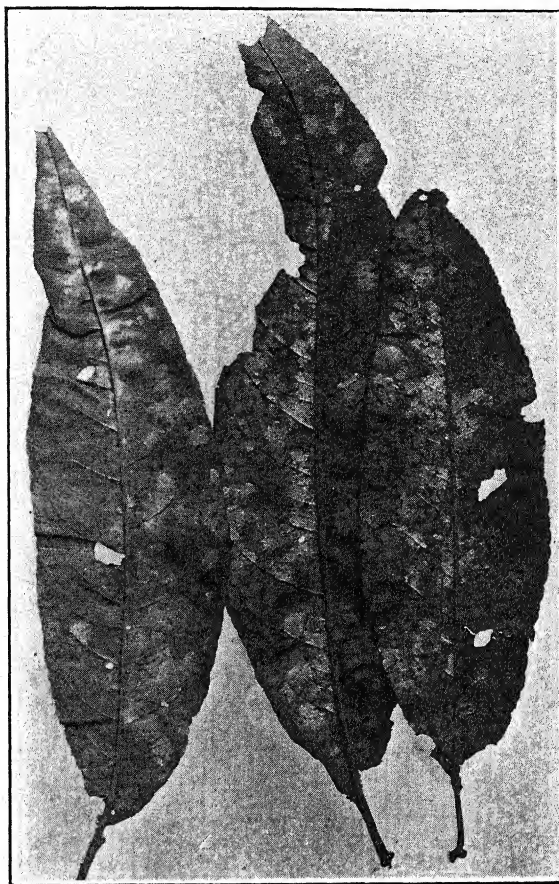


FIG. 1. Frosty mildew of peach on the lower surfaces of the leaves.

become reddish. The whitish patches may be few and remain discrete, or may become so numerous as to occupy a large proportion of the lower leaf surface. Affected leaves are shed prematurely. Prior to defoliation, the lesions may become necrotic and the tissues brown. The severity of this defoliation appears to be correlated with the abundance of the lesions.

According to the records in the Division of Mycology and Disease Survey, U. S. Department of Agriculture, frosty mildew has been collected in Alabama, Arkansas, Connecticut, Delaware, Florida, Georgia, Illinois, Louisiana, Maryland, Mississippi, Missouri, North Carolina, Ohio, South Carolina, Texas, Virginia, and Washington. Apparently, it occurs only in neglected orchards and among young trees in nurseries, and is not encountered in well-cared-for orchards. The exsiccati and records of collections of European mycologists indicate that *Cercospora persica* is widespread throughout Europe and also throughout the Orient.

*Conidial Stage.* The essential structural features of the conidial stage can best be discerned from free-hand sections of lesions cut parallel to the leaf surface. Such sections show that the whitish coating is imparted to the leaf by a superficial, tangled, mycelial mat that bears a profusion of conidia. The superficial mycelium emerges from the stomates and develops a much-branched, closely appressed weft. Short, knob-like, lateral branches on this mycelium constitute the conidiophores. Each conidium is abstricted from the apex of a conidiophore, is dislodged as it matures, and the conidia thus accumulate in masses. The conidia are hyaline, vermicular to clavate, several-septate, and range in size from  $17-86 \times 2.5-7 \mu$ . Conidia from fallen leaves may be brownish, and occasional longitudinally oriented septa may be formed.

*Perithecial Stage.* During September the spermogonia and perithecial primordia begin to appear concurrently within the infected tissues. Each lesion comes to be thickly beset, on the lower leaf surface, with these structures. They are intermingled and appear as punctiform, black bodies. Examination discloses that the spermogonia arise as spherical masses of closely interwoven hyphae. Their interior becomes spermatiferous and the outer hyphae coalesce to form a thin, dark brown, membranous wall. Eventually, the spermogonia protrude slightly above the leaf surface, and an aperture is formed through which the spermatia are liberated. They mature soon after the leaves have fallen and the rod-shape spermatia,  $2.5-4 \times 0.5 \mu$ , are produced abundantly during a period of approximately 2 months. Spermogonia and perithecial primordia appear to have been observed in 1891, by Smith (4, pp. 91-92). He states that "very late in the season before the fall of the leaves or afterward, pycnidia develop on the conidial surface in such a manner as to lead one to believe them a part of the cycle of development. . . . Other bodies similar to the phoma conceptacles but destitute of spores accompany these, and a search toward spring would perhaps reveal the presence of ascospores, and might lead to the determination of the true position of this form-genus."

The perithecial primordia are indistinguishable in external appearance from spermogonia. If material is collected at intervals throughout the fall, however, and sectioned in paraffine, it will be found that, after spermatial formation has ceased, the spermogonia are empty, whereas the perithecial stromata remain compact and their innermost portions consist of cells having deeply-staining contents.

Peach leaves in contact with the soil rather quickly disintegrate. If they are kept from direct contact with the soil, however, by the interposition of a layer of dry grass or other suitable material, the perithecial stromata will have become transformed into mature perithecia by late April or early May. If such overwintered material is examined with a hand lens, small patches,



presumably the sites of the lesions formerly occupied by the conidial stages, or else large portions of the lower surface of the decaying leaves, are seen to be densely occupied by prominent black objects. Microscopic examination reveals that these objects are perithecia. They are globular and open to the surface by a perforation through the papillate apex. They vary in size from  $75-100 \times 60-106 \mu$ . Their wall is membranous, being composed of a thin layer of cells with brown walls. The asci are fasciculate as shown in preparations obtained by maceration. They are elongate-saccate to clavate, lack an apical pore, and measure  $36-55 \times 7-10 \mu$ . Paraphyses are lacking. The ascospores are biserially arranged, are unequally bicellular, slightly curved, hyaline, and measure  $12-20 \times 2.5-3.5 \mu$ .

*Genetic Relationship of Conidial and Perithecial Stage.* Genetic connection between the well-known conidial stage of this organism and the ascigerous stage has been demonstrated by cultures and by inoculations on peach leaves.

Both conidia and ascospores germinate readily in water or on various artificial media. The colonies that arise from conidia are similar in every respect to those produced from ascospores. The fact that conidia are produced abundantly in 3- to 4-day-old cultures initiated by sowing either conidia or ascospores is perhaps of more significance. These conidia are indistinguishable in size, color and septation, and in the manner of their formation from those borne on naturally inoculated leaves.

Small peach trees of the varieties Elberta and Belle of Georgia, grown in the greenhouse, were employed for the inoculation tests at Durham, N. C. The inoculum consisted of a suspension of conidia either (a) from diseased leaves collected in the orchard, or (b) from conidia from cultures initiated from conidia, or (c) from conidia from cultures initiated from ascospores. The inoculum and leaf surfaces were kept moist by strips of paper towels serving as wicks. One end of these strips was inserted in jars containing water and the other was applied to the inoculated leaves. Approximately 3 weeks after inoculation, lesions bearing a whitish coating, the *Cercospora* stage, were present on all inoculated leaves. Examinations of this coating revealed that it was morphologically identical with that occurring on trees in orchards parasitized by *Cercospora persica*.

At Experiment, Georgia, on June 2, 1936, 50 leaves on 2 greenhouse-grown, seedling, peach trees were inoculated with conidia, suspended in water, from a single ascospore culture. The entire twigs bearing these leaves were then wrapped with absorbent cotton, which was moistened twice daily for 3 days, after which the cotton was removed. At the same time and as a control, 2 twigs bearing 28 leaves were wrapped with absorbent cotton and moistened in the same way. On June 20, pale spots were evident on some of the inoculated leaves. On July 8, 37 of the leaves showed abundant infec-

tion, with typical *Cercospora* conidia on the undersurface of the leaves. No infection occurred on the noninoculated check leaves. Spermogonia and young perithecia were found on these inoculated leaves during the succeeding fall.

At the same time, portions of overwintered peach leaves bearing mature perithecia were bound to the lower surface of 32 leaves on field-grown peach seedlings on which frosty mildew was absent during the previous year. Twenty of the leaves thus inoculated developed typical frosty mildew.

*Taxonomy.* The genus *Cercospora*, with *Cercospora persica* as the type, was created by Saccardo (2, p. 20) in 1880, to include hyaline forms that previously had been included in the genus *Cercospora*. He characterized the genus as follows: "Tota candida, biogena, Hyphae simplices vel ramulosae, hyalinae. Conidia vermicularia, pluriseptata, hyalina. Est Cercospora Mucedinea." Such a characterization is manifestly inadequate, as anyone knows who has critically examined several species of *Cercospora*. It should be emphasized also that a proper understanding of the morphology of *Cercospora persica* apparently was first had as a result of the investigations of Tsuji (5). He noted that the fruiting hyphae emerge from the stomates, form a prostrate web external to the leaf, and bear the conidia upon short conidiophores arising from the superficial fruiting hyphae. He also noted that the conidia, although hyaline at first, may become colored, with age, an observation that accords with our findings. It may be recalled that the dematiaceous genus *Clasterosporium* possesses phaeophragmous conidia borne in this manner, and that Tsuji, therefore, applied the name *Clasterosporium persicum* (Sacc.) Tsuji to this pathogen on peach.

It appears highly probable that all other fungi that produce white patches on leaves and bear conidia like the peach pathogen, have the same cycle of development and possess a similar perithecial stage. This assumption is supported by the junior writer's findings with *Mycosphaerella arachnoidea* Wolf (*Cercospora arachnoidea*) on mulberry (7), with unpublished investigations in progress on *Cercospora cana* Sacc. on *Erigeron* spp., and with Demaree and Cole's (1) results with *Mycosphaerella caryigena* Dem. and Cole (*Cercospora caryigena* (E. and E.) Höhn.) on pecan. Demaree and Cole were unable to demonstrate conidiophores in the pecan downy-spot fungus, but there would appear to be little doubt that they are of the type possessed by the peach pathogen.

Other fungi now included in *Cercospora* possess similar developmental cycles, but bear fascicled conidiophores, as is the case in *Mycosphaerella mori* (Fekl.) Wolf (6). Some investigators might choose to apply a different generic name to the conidial stage of such organisms. It seems a more reasonable procedure, however, to revise and enlarge the Saccardoan concept of *Cercospora* so as to include such forms.

The segregation of genera on the basis of whether the conidia are hyaline or colored, as in *Cercospora* and *Clasterosporium*, appears to be an unfortunate one, at least with some of the species. This is not an isolated situation, since it is the experience of all mycologists that there are genera possessing hyaline conidia some of whose species become beautifully colored, with age, or under cultural conditions. It seems advisable, at least until complete life histories are known, to avoid any wholesale shifting of specific names from one imperfect genus to another. It seems to the writers, also, that confusion would be lessened, and it would be otherwise advantageous to continue to regard *Cercospora persica* as the type of the genus *Cercospora*.

The form and structure of the perithecial stage, the asci, which tend to remain in a fascicle when removed from the perithecium, the absence of paraphyses, and the hyaline, 2-cell ascospores show that the ascigerous stage of the peach pathogen belongs in the genus *Mycosphaerella*. Although this genus is one of the largest among the Ascomycetes, it is a curious fact that none of its members previously have been reported to occur on peach. Whether *M. sentina* (Fr.) Schröt. occurs on peach as reported by Seymour (3) is not indubitably established. Since the perfect stage has not been described, the name *Mycosphaerella persica*, n. sp. is proposed and is briefly characterized as follows:

***Mycosphaerella persica*, n. sp.**

Perithecia vulgo hypophylla, plus minusve dense dispersa, in greges numerosos vel per totum folium distributos, punctiformia, nigra, erumpenti-immersa, globosa, ca. 75–110 × 60–106  $\mu$  diam.; ostiolo tantum papilliformi vel obtuse conico; pariete membranaceo, parenchymatico, pellucide atro-brunneo. Asci cylindracei-clavati, breviter stipitati, octospori, paraphysati, 36–55 × 7–10  $\mu$ . Sporae distichae, rectae vel curvulae, inaequaliter septatae, vix vel lenissime constrictae, loculo superiore paulo crassiore, hyalinae, 12–20 × 2.5–3.5  $\mu$ .

Spermogoniis autumnno efformantibus, dense aggregatis, plerumque hypophyllis, innato-erumpentibus, ovatis vel globosis, atris, 48–72 × 52–91  $\mu$ , spermatis bacilliformibus, hyalinis, 2.5–4 × 0.5  $\mu$ . Hab. in foliis dejectis Pruni persicae.

Status conidicus: Statum conidicum *Cercospora persica* sistit. Laesionibus indeterminatis; superiore flavescentibus, infra candidis; conidiophoris mycelio fertile arachnoideo oriundis, hypophyllis, bursiformibus, apice conidia gerentibus; conidiis quoad magnitudinem variabilibus, plerumque vermicularibus vel clavatis, solitarie oriundis, sed mox secendentibus, raro guttulatis, pluriseptatis, hyalinis, deinde rarissime dilute brunneis, 17–86 × 2.5–7  $\mu$ . Hab. in foliis adhuc vivis Pruni persicae.

Syn. *Cercospora persica* Sacc. Fgi. Ven., nov. ser. V: p. 189, Sacc. Michelia I: 88; Sacc. Fgi. Ital. del. Tab. 67: Hedwegia 15: p. 119; Thüm. Litor No. 247.  
Sicc. Rab. F. E. No. 2151 and No. 3081; Sacc. Mycoth. Ven. No. 598; Thüm. M. U. No. 1568; Buchholtz and Bondarzen Fgi. rossici No. 645.

*Cercospora persica* Sacc. Syll. Fung. 4: p. 218, 1886. Sicc. Roumg. Fgi. Gall. No. 4987.

*Clasterosporium persicum* (Sacc.) Tsuji. Ann. Phytopath. Soc. Japan, v. 1, No. 2, 23–35. 1919.

For the convenience of mycologists, specimens of both the conidial and ascigerous stages have been deposited in the Farlow Herbarium, Harvard University, the Mycological Collections, U. S. Department of Agriculture, the herbarium of Duke University, and that of the Georgia Experiment Station.

#### SUMMARY

The morphology and cycle of development of the fungus generally known as *Cercospora persica* Sacc., causing frosty mildew of peach, has been studied. These investigations have shown that, in addition to the conidial stage, this pathogen produces spermogonia and perithecia.

The conidial stage was made the type of the genus *Cercospora* and it appears desirable thus to retain it and to enlarge the Saccardoan concept of its generic limits.

The perithecial stage appears never to have been observed previously. It belongs to the genus *Mycosphaerella*, and the peach fungus is accordingly given the name *Mycosphaerella persica*, n. sp.

Both spermogonia and perithecia are initiated simultaneously during the fall. Spermatial production ceases after approximately two months while the perithecia do not mature until the following spring.

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## THE POSSIBILITY OF INSECT TRANSMISSION OF ALFALFA DWARF<sup>1</sup>

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In a recent article evidence was presented<sup>2</sup> showing that the dwarf disease of alfalfa is of a virus nature. It is fairly readily transmitted by any grafting method resulting in a union of healthy and diseased tissue. Experiments show that dwarf spreads readily from diseased to nearby healthy plants, but the exact method by which the causal agent is carried has not been demonstrated. The possibility that some insect or insects may be responsible for transmitting the disease was suggested by the results of some preliminary experiments conducted in California, herein presented as matters of record, since this project has been discontinued.

### SPREAD OF DWARF IN PLOTS SUGGESTS INSECT TRANSMISSION

In one of these experiments healthy alfalfa seedlings, grown in mountain soil in pots in the greenhouse, were planted in 4 rows on a small piece of land adjacent and parallel to a badly diseased plot of alfalfa. The following seed lots of alfalfa were used: Turkistan (F.C. 19316), Persia (F.P.I. 86361), (Iran) Persia (F.P.I. 86362), Turkey (F.P.I. 100723), Arabia (F.P.I. 98363), California Common, Spanish and Turkistan (F.C. 19304). None of the plants was over 15 feet from diseased plants, and most of them were much closer. The land on which these rows were planted had grown alfalfa the previous year, hence the possibility of the disease being carried in the old roots left in the soil must be considered. An average of 34.2 and 97.6 per cent of all of the plants were diseased at the end of the first and second years, respectively. There was no significant difference in the amount of disease in the different lots of plants. The possibility of seed transmission is eliminated, since most of the seed came from foreign countries; therefore, it seems that the high percentage of disease must be attributed to infested soil or to insect transmission. Previous experiments dealing with soil transmission suggest that alfalfa dwarf does not live in the soil, hence the only plausible explanation for the rapidity of its spread through these 4 rows is that it was carried from the adjacent badly diseased plot by insects.

Another experiment, in which 4 rows of healthy alfalfa seedlings were planted beside 2 other rows, containing both diseased and healthy plants, also indicates that dwarf is insect-transmitted. The plants were set out in

<sup>1</sup> Received for publication Jan. 8, 1937. Cooperative investigations of the Division of Agronomy, California Agricultural Experiment Station and the Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

<sup>2</sup> Weimer, J. L. Alfalfa dwarf, a virus disease transmissible by grafting. Jour. Agr. Res. [U. S.] 53: 337-347. 1936.

early spring, by late autumn 7 dwarfed plants were found in the row adjacent to that containing diseased plants, 10 in the next row away from the latter, 4 in the third row, and none in the fourth. No dwarfed plants occurred in the ends of these rows beyond the diseased plants and none in numerous basins in the vicinity, but somewhat farther away. By the middle of the second year the disease had spread considerably in the 3 rows and had reached the fourth, as well. At no place, however, had it spread more than about 25 feet from the diseased plants, transplanted at one end of the plot. This suggests that the disease is insect-transmitted and that it may not spread from a given center more than 25 feet or thereabouts in  $1\frac{1}{2}$  years. This, however, is only a single instance and merely shows rate of spread under this one set of conditions. The direction of spread was against that of the prevailing winds. No disease had developed in plants growing in the basins near these rows during 2 years prior to the initiation of this experiment and in the 2 years it was in progress. This shows that the soil did not contain the causal agent of dwarf and that the latter was not being brought in by wind, irrigation water, or other agent. The disease must have come from the diseased plants in the two adjacent rows, and there seems to be no logical explanation of how it was carried except by insects. An insect that is a very weak flier, does not fly at all, or can transmit the disease only in the wingless stage is suggested both by the short distance the disease spread in this experiment and by the fact that it did not spread more readily from pot to pot in other experiments.

#### CAGE EXPERIMENTS

In the spring of 1934 a portion of 2 plots of very badly diseased alfalfa (practically 100 per cent dwarfed) was plowed. A strip of old alfalfa was left on each side of the plowed areas as a source of inoculum. Three insect cages, approximately  $2' \times 3' \times 12'$ , were placed about equidistantly along the length of each plot. Three cages were covered with 80-mesh muslin, except that the tops of 2 were covered with celloglass. The ends of one of the cages with a celloglass top were left open to permit the entrance of insects and yet supply a somewhat comparable amount of shading for the plants. The other 3 cages were covered with 40-mesh muslin except that the ends of one were left open. The soil beneath 3 of the cages was fumigated with carbon bisulphide.

A section of soil of corresponding size outside the cages was treated with  $CS_2$  as a control on the possible injury to the plants or to the causal agent of alfalfa dwarf. No such injury occurred. Fifty healthy alfalfa plants of the Arizona common variety, grown in the greenhouse, were planted in each cage and a row was planted with some of the same plants on each side of each cage, the full length of the plot, as well as between the cages. The

plants grew well both inside and outside the cages, but by autumn the size of the roots of the outside plants averaged nearly twice that of the plants within the cages. This held true throughout the experiment, the final average measurements of the diameters of the roots, near the crowns being: Caged plants, 11.37 mm.; not caged, 20.25 mm.; caged, but ends of cages open, 10 mm.; and not caged, soil treated with CS<sub>2</sub>, 18.46 mm. The celloglass top of the 2 cages made no difference as far as observed or as was indicated by the size of the roots at the end of the experiment. Growth of the plants under the cages with open ends was little if any better than that under the closed cages; in fact, their roots measured slightly less in diameter. The plants were set out on April 26, 1934, and the final data were taken July 15, 1935. The plants were all dug, the bark removed from the tap roots, and the presence or absence of dwarf determined. The data, recorded in table 1, show no infection in the plants under the 4 closed cages, 42.56 per cent in the plants outside, and 12 per cent under the cages with the ends open. The diseased plants under the open-end cages were all under the one with the cloth top. The fact that no disease appeared under the closed cages and did occur abundantly outside appears to confirm the supposition that dwarf is insect-transmitted. However, it is difficult to explain why no disease appeared under the one open-end cage. It may be that the insect responsible for the transmission of dwarf failed to find its way into the cage (an explanation that does not seem plausible), or that possibly light or temperature under the cage with the celloglass roof was unsuitable for either the insect or the development of the disease.

TABLE 1.—*The effect of plant caging on the development of alfalfa dwarf*

Treatment given plants	Number of plants		Percentage of infection
	Total	Infected	
Duration of experiment April 26, 1934, to July 15, 1935			
Caged .....	200	0	0.00
Not caged .....	813	346	42.56
Caged, ends of cages open .....	100	12 <sup>a</sup>	12.00
Not caged, soil treated with CS <sub>2</sub> .....	44	14	31.82
Duration of experiment Feb. 15, 1935, to July 31, 1935			
Caged .....	291	0	0.00
Caged, ends of cages open .....	125	0	0.00
Not caged .....	1155	6	0.52
Caged: A. Grafted .....	24	11	45.83
B. Seedlings .....	21	0	0.00
Caged: A. Diseased plants planted among seedlings...	12	12	100.00
B. Seedlings .....	181	4	2.21
Not caged: A. Grafted with diseased scion .....	25	14	56.00
B. Grafted with healthy scion .....	25	0	0.00

<sup>a</sup> All in one cage, making 24 per cent in that cage, and none in the other. The cage under which no disease developed was the one covered with celloglass.

This experiment with some modifications was duplicated in 1935. Five cages of the same size covered with 50-mesh muslin were used. The ends of one cage were left open as a control. The soil beneath all of the cages was fumigated with  $CS_2$ . On February 15, 1935, alfalfa seed was sown in rows inside the cages and one row on each side of them and about one foot away to serve as controls. Many old diseased alfalfa plants were present on each side of the row of cages.

Certain additional features were incorporated into this experiment: Twenty-five grafted plants were set beneath a sixth cage of like construction and placed near the others, so designed as to show whether dwarf would develop beneath these cages. A segment of diseased root was grafted to the tap root of each healthy plant, as explained in a previous paper.<sup>3</sup> Twenty-five additional healthy plants, grafted with segments of healthy roots and a like number grafted with portions of diseased roots were planted outside of the cages. Likewise, 20 diseased plants from the field were freed of all débris, dipped into a concentrated solution of nicotine and soap, and set among the healthy seedlings in one of the other 5 cages to determine if the disease would go to the healthy plants in the absence of insects.

The experiments were discontinued on July 31, 1935, and the plants were dug and examined. The data obtained are given in the lower section of table 1. Here, again, no disease developed under the cages beneath which only healthy plants were growing. Six plants out of 1,155, or 0.52 per cent, of the plants growing in the rows outside the cages showed dwarf root symptoms at the time the experiment was discontinued. Slightly more than 45 per cent of the grafted plants growing in one cage became diseased, thus showing that conditions under the cages favored dwarf development. Fourteen of the 25 plants grafted with diseased root segments and planted outside of the cages, or 56 per cent had dwarf, while the 25 healthy plants, grafted with healthy tissue, remained healthy. Likewise, 4 plants out of 181, or 2.21 per cent, growing under a cage in close proximity to diseased plants, became infected. How the disease was transmitted from the diseased to the healthy plants under the cage is not definitely known. It is possible, however, that some insect capable of transmitting the disease may have gained access to the cage, possibly through the soil or on the diseased plants, having escaped destruction when the plants were treated with nicotine just before being planted in the cage. The small amount of infection in the plants outside of the cages is due to the short duration of the experiment.

Continuation of this experiment for another year or longer would have been profitable, for it would have allowed time for more disease to develop. The general trend of the results of this experiment, however, was the same as that of the experiment previously described.

<sup>3</sup> Weimer (See footnote 2).



During the summers of 1934 and 1935, several experiments were conducted in which different insects were caged on dwarfed plants for 3 or more days and then transferred to healthy plants under cages, where they were left for several days or even weeks, in some experiments, and then the plants were dusted with tobacco. In several cases sweepings were made in alfalfa fields with an insect net and all of the insects obtained were placed in 2 cages ( $3 \times 3 \times 3$  ft.) containing both dwarfed and healthy plants and allowed to remain there throughout the experiment or about 4 months. The insects could move freely from diseased to healthy plants. All of the cages were held out of doors exposed to maximum daily temperatures varying from  $80^{\circ}$  to  $110^{\circ}$  F. Sometimes insects were taken from diseased plants growing in the field and placed on healthy plants under cages. In some cases the plants were held after inoculation for several weeks and in others for several months. In no case was there any evidence that dwarf had been transmitted by means of these insects under the conditions provided in these experiments. Among the insects tested were the following:

The pea aphid (*Macrosiphum Illinoia pisi* Kalt.), the bean aphid (*Aphis rumicis* L.)<sup>4</sup>, a species of thrips, probably *Frankliniella occidentalis* Perg., several species of leaf hoppers (*Draeculacephala minerva* Ball, *Gypona angulata* Spbg., *Aceratagallia californica* Baker, *A. obscura* Oman, and *Deltocephalus* sp.), the three-cornered alfalfa hopper (*Stictocephala festina* Say), 2 species of cucumber beetles (*Diabrotica balteata* Lec.) and *D. soror* Lec.) a plant bug (*Lygus hesperius* Knight) and a sowbug (*Porcellio laevis* Latr.)<sup>5</sup>.

#### GENERAL DISCUSSION AND CONCLUSIONS

Healthy plants set in rows in the field adjacent to rows of dwarfed plants, but not in direct contact with the latter, became diseased rather rapidly, while control plants, farther from the diseased ones, remained healthy. The plants nearest those affected with dwarf usually became diseased most promptly. In general, however, the disease spreads in a more or less random fashion, typical of insect-transmitted viroses. No logical explanation for the manner of spread of dwarf in some of the writer's experiments is apparent unless insects serve as the agents of transmission.

Caged plants growing in the field remained dwarf-free, while plants of the same lot growing outside of the cages became diseased. Grafted plants growing under cages in the field developed dwarf, thus showing that conditions under the cages were not unfavorable for the disease. It would appear, then, that the exclusion of the insects from the caged plants was responsible

<sup>4</sup> Determined by T. L. Bissell, Entomologist, Georgia Agricultural Experiment Station, Experiment, Ga.

<sup>5</sup> The leaf hoppers were determined by P. W. Oman, the *Diabrotica* sp. by H. S. Barber, the species of *Lygus* by H. G. Barber, all of the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, and the sowbug by J. O. Maloney, Division of Marine Invertebrates, U. S. National Museum.

for the latter remaining healthy. However, when different species of insects commonly found on alfalfa were caged on diseased plants and then on healthy ones, the latter did not become infected. The cage experiments have been more or less preliminary, and more work must be done before the insect, or insects, responsible for transmitting dwarf is found or before it can be concluded that the disease is not insect-transmitted.

#### SUMMARY

Observations made on the spread of alfalfa dwarf in the field suggest that the disease is insect-transmitted.

Caged alfalfa plants growing near infected plants did not become dwarfed, while uncaged plants did become dwarfed.

None of the insects tested transmitted the dwarf disease to alfalfa plants under the conditions of the experiments.

### THE OCCURRENCE OF AERIAL BACTERIAL STRANDS ON BLOSSOMS, FRUITS, AND SHOOTS BLIGHTED BY *ERWINIA AMYLOVORA*<sup>1</sup>

S. S. IVANOFF AND G. W. KEITT

(Accepted for publication March 1, 1937)

In connection with studies on the epidemiology of fire blight, blossoms of potted pear trees (*Pyrus communis* L.) were inoculated in the greenhouse with the fire-blight organism, *Erwinia amylovora* (Burr.) Bergey *et al.* Soon after the first symptoms appeared some very fine, hair-like, slightly curved, glistening structures were noticed among the pedicel hairs of diseased blossoms. Further examination showed that these strands were neither normal host structures nor chance extraneous deposits, but resulted from the pathological processes within the plant, apparently being made up of bacterial cells bound together by a cementing substance. Since the occurrence of these structures seems not to have been reported in the literature,<sup>2</sup> and since, under certain conditions, they may possibly play a rôle in the epidemiology of fire blight, it was thought worth while to investigate them further. The studies made so far on these strands are the subject of this paper.<sup>3</sup>

<sup>1</sup> Approved for publication by the Director of the Wisconsin Agricultural Experiment Station.

<sup>2</sup> The occurrence of similar strands or "cirri" has previously been reported on young mulberry shoots inoculated in the greenhouse with *Phytomonas mori* (Boyer and Lambert amend E. F. Smith) Bergey *et al.* (Cf. Smith, E. F. Bacteria in relation to plant diseases. Carnegie Inst. Wash. Pub. 27, V. 2. 1911). Similar structures also have been observed by one of us on corn culms and leaves inoculated in the greenhouse with *Phytomonas stewartii* (E. F. Smith) Bergey *et al.*, the plants having been grown under high temperature and relative humidity. In both of these cases, however, the strands were heavier and thicker, and consequently more conspicuous, than those produced in connection with fire blight.

<sup>3</sup> Ivanoff, S. S., and G. W. Keitt. Aerial bacterial strands in fire blight (Abst.). Phytopath. 27: 132. 1937.



FIG. 1. Aerial bacterial strands on pedicels of Bartlett pear blossoms inoculated with *Erwinia amylovora*.  $\times$  approximately 3. A. Blossom cluster showing numerous strands, e.g., at a and b, and two drops of bacterial exudate near the base of the pedicels. B. Unusually long strands on a pedicel.

*Description.* The appearance of the strands (Fig. 1, A and B) is such that unless they occur in unusually large numbers or size, as is sometimes the case, they could easily be overlooked. Their length varies from a fraction of a millimeter to several centimeters. In most cases, however, it exceeds only slightly the length of the hairs of the pedicel. The strands vary in thickness from about 8 to 45  $\mu$ , being on the average slightly thinner than hairs of the pear pedicel (Fig. 2, F and G). They are usually cylindrical throughout most of their length, with the exception of the free end, which may gradually taper off. In some less common cases, however, they have small swellings at more or less regular intervals. Such strands resemble the viscid silk produced by some spiders of *Aranea* spp.<sup>4</sup> However, the bacterial strands can easily be distinguished from these and other structures by methods to be mentioned later. Ordinarily, the strands are slightly curved but in some cases they form loops or irregularly curved structures. Under high magnification the surface of the strands appears smooth, the interior granular. The thin strands are usually colorless, and glistening, but the thicker ones appear yellow to light brown. When viewed under the microscope as they lie dry on a slide, the strands appear to have thick walls (Fig. 2, F and G), but this is an optical illusion. Under some conditions the strands are very brittle, breaking off easily upon impact with a cold object. However, they feel sticky and tenacious when touched with a warm hand.

*Structure.* The structure of the strands was investigated by microscopic and cultural technique. When placed in a drop of water the strands disintegrated instantly. The drop became clouded by bacteria, with no trace of the former strand or other structure in any way resembling it. In similar tests it was found that older strands that had been kept dry disintegrated in water a little more slowly than those freshly formed, and left traces of sticky material on the glass slide. A loopful of the clouded liquid, plated out in nutrient dextrose agar, yielded thousands of bacterial colonies, which subsequent inoculation tests showed to be the fire-blight pathogen. If the strands are placed in a drop of glycerine instead of water, the disintegration occurs more slowly and can be observed in detail under the microscope (Fig. 2, A-D). Additional proof that these strands are composed of fire-blight bacteria and a cementing substance was obtained by placing some of them on a clean, sterile glass slide and blowing moist air on them through the mouth. The moist air caused partial disintegration of the strands. Examination of such partly disintegrated structures under high power or oil immersion showed distinctly that they are composed of bacteria and a cementing material. The nature of this cementing substance has not been investigated.

<sup>4</sup> Comstock, J. H. The spider book. 721 pp. Doubleday, Page and Co., Garden City, New York. 1912.

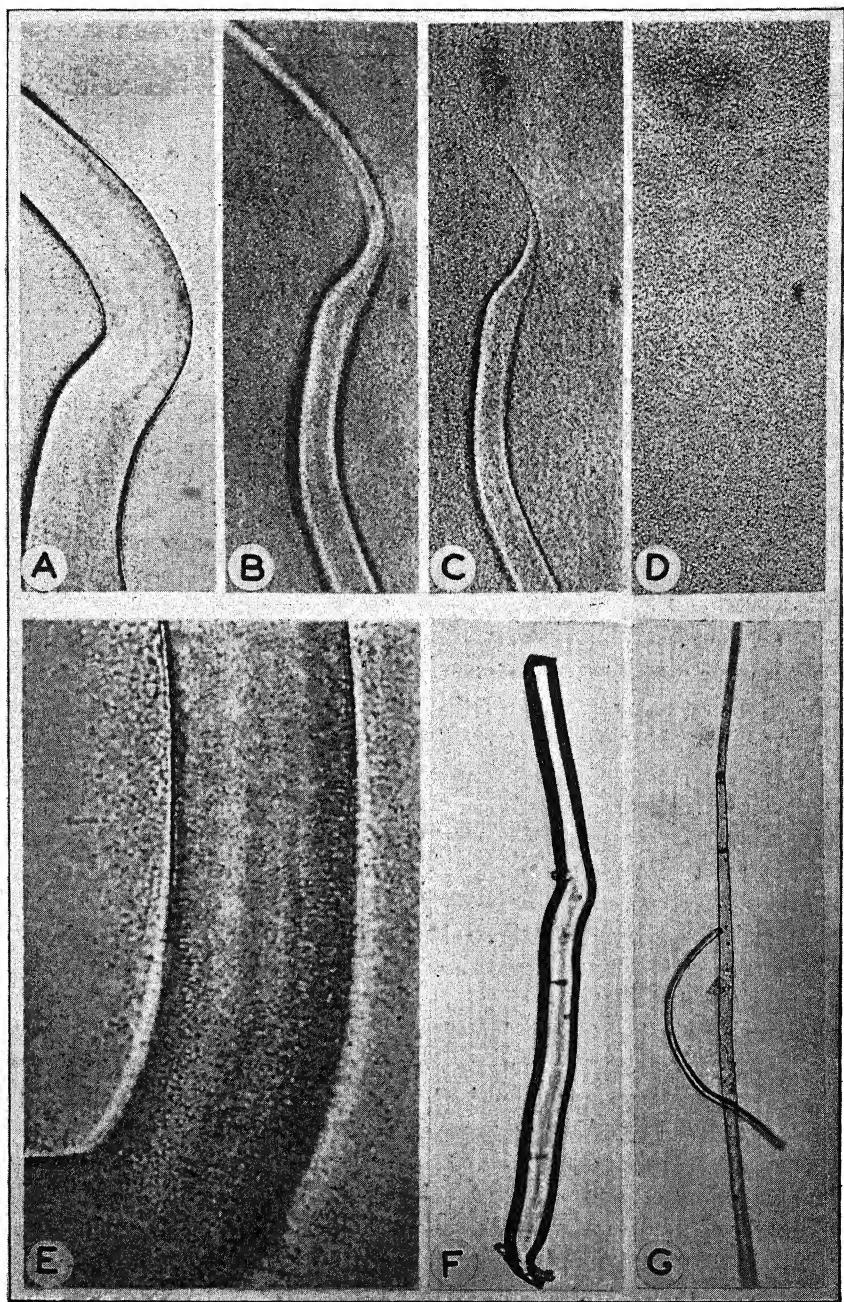


FIG. 2. A-D. Stages in the disintegration of a bacterial strand in a drop of glycerine.  $\times 260$ . A few minutes after suspension in glycerine. Bacteria are beginning to be liberated from the strand. B. Two hours later. There has been partial disintegration of the strand, and great numbers of bacteria are suspended in the drop. C. Four hours later. D. Six hours later. The strand has completely disintegrated. E. Enlarged portion of A.  $\times 550$ . F. A relatively thick bacterial strand.  $\times 75$ . G. A hair from the pedicel of a pear blossom with a portion of a curved bacterial strand of typical thickness adhering to it.  $\times 75$ . F and G were photographed as the subjects lay dry on glass slides.

*Infectivity.* The infectivity of the strands was tested by removing the tips of several of them with aseptic precautions and placing these portions in the receptacle cups of pear and apple blossoms. Five such sections were placed in the blossoms of an apple tree that had previously been kept in a moist chamber overnight. The tree bore about 40 more blossoms that served as controls. Each of the blossoms containing the strands developed fire blight in 5 days, and certain of them produced drops of exudate on the seventh day after inoculation. All the uninoculated blossoms remained healthy. On another occasion 5 strand sections were placed in the receptacle cup of pear blossoms in the field. Only the inoculated blossoms became infected. Strand sections were found to contain viable and infective bacteria after having been kept in sterile Petri dishes for 7 days at 20° C.

*Differentiation from Structures of Similar Appearance.* In searching for these strands in the field it was realized that they could easily be confused with other structures of plant or animal origin that naturally occur or chance to be deposited on the host, such as hairs of the host, cotton or hemp fibers, spider webs, and down from cottonwood (*Populus* spp.) seeds. The hairs abundantly produced on the pedicels of the host are probably chiefly responsible for these strands not having been discovered previously. Microscopic study of the various structures that might be mistaken for the bacterial strands reveals characters that readily differentiate them; but it is unnecessary to discuss these characters, since a simple test easily distinguishes the bacterial strands from all the others. When placed in a drop of water, the bacterial strands quickly disintegrate, releasing myriads of bacteria, while all the others retain their form.

*Origin.* The place of origin of these strands has been studied thus far only by microscopic observations of untreated material. In many cases it was difficult to follow the strand to its place of attachment because of the obscuring effect of the numerous hairs on the pedicel. In a few cases, however, by use of the "Ultrapak" microscope, the strands were seen protruding through small openings in the surface tissue. Assurance that at least some of these strands were not deposited upon the host in some way from the outside or formed by stringing out the well-known drops of bacterial exudate by some natural agent was obtained through an experimental study reported in the following paragraph.

*Manner of Development.* The manner of development of the strands was made the subject of a simple study. Six pear-blossom clusters showing only slight symptoms of blight, but each bearing one or more visible strands on the pedicel, were examined microscopically for the number and position of the strands on each pedicel, for freedom from any bacterial exudate in the form of drops, and for freedom from insects or any other conspicuous foreign material. The approximate place of origin of each strand was marked



without wounding the tissue, after which the strands were removed with forceps and the clusters placed with the cut ends of the subtending twigs in water and left in the laboratory overnight. On the following morning the clusters were examined and found to bear strands at or near the places where the removed strands formerly stood. A similar test, performed in the greenhouse without removing the blossoms from the tree, gave like results.

Additional evidence of the progressive development of strands was obtained by actually measuring the gradually increasing length of one at suitable time intervals. The particular strand measured was one of unusual length. At about 9:00 a. m. it was cut to a length of approximately 4 mm. At about 4:00 p. m. of the same day it measured 9 mm., and 24 hours later it was about 20 mm. long. By this time the strand was irregularly curved, similar to the one shown in figure 1, B.

*Conditions Affecting Development of the Strands.* The conditions under which the strands develop have not been comprehensively studied. When these structures were first observed the temperature of the greenhouse was being maintained at about 22° C., while the relative atmospheric humidity varied from 62 to 80 per cent. The temperature and relative humidity in the field, where some of the strands developed following artificial inoculation, varied from about 10° to 27° C. and about 47 to 100 per cent, respectively. Relative humidities of about 35 and 95 per cent, respectively, were artificially maintained under bell jars in the laboratory, in connection with some tests in which inoculated blossoming twigs of pears and apples were kept with the cut ends in water for several days. Although drops of exudate were formed on some of the inoculated blossoms, no bacterial strands were noticed. It has been noted on many occasions that not every inoculated pear strands than others. It seems probable that both atmospheric conditions and blossom produces strands, and that some blossoms produce more and longer those within the plant exert important influences on strand formation.

*Hosts on which the Strands have been Produced.* Several thousand bacterial strands have been observed in the course of these studies in connection with artificial inoculations made at various times on the blossoms of pears in the greenhouse and in the field, on young fruits and shoots of pears in the field, and in two cases on the pedicels of crab apple (*Pyrus coronaria* L.). The inoculations were made by either depositing a drop of the bacterial suspension in the receptacle cup of the flower or by puncturing the pedicel. The strands were most easily produced on pear blossoms and less so on the fruits and shoots. On some individual young pear fruits, however, more than 50 short strands have been seen, most of them in the shape of short drum sticks, together with the numerous droplets of exudate. It should be noted that, although a great number of strands has been produced on pears by artificial inoculation, none has yet been produced on the apple. So far,

these strands have not been found on blossoms, shoots, or fruits infected naturally; but the search for them on apple has not been exhaustive, and there has been no opportunity to seek them on naturally infected pear.

*Dissemination.* The disappearance of these strands from the host structures has been noted. After a light rain, as would be expected, they were no longer found on blossoms where they had formerly been observed. But the disappearance of strands in the absence of rain also has been noted. The possibility that they may be easily broken off from their place of origin and disseminated by the wind is obvious. An incident in the greenhouse gave evidence that this happens. A pear tree with a number of strand-bearing blossom clusters was being examined in the greenhouse hallway. A number of unusually long strands that were present on a diseased cluster were about to be cut off and used in some of the tests. A door was unexpectedly opened, air rushed in, and the strands were carried off.

The dissemination of the strands by wind in nature, which may possibly be of importance in the epidemiology of fire blight, was further tested in the following manner. Some blossoms were inoculated, and as soon as the strands began to appear glass slides provided with holes near the edge and smeared with glycerine were hung around the infected clusters. Seven selected clusters were used in the test, each having 2 slides hung near it in such manner that they could not touch the blossoms. There were gentle breezes for 2 days, during which time the glass slides were examined periodically. Since the strands disintegrate only slowly, even in an abundant amount of glycerine, those that might lodge on the slide during the night were expected to adhere until it should be convenient to examine them. One strand was found on a slide in the evening, six hours after the experiment was started. On the following morning 2 more were found, and in the evening of the same day, 2 more, making a total of 5 caught during the experiment. These strands became partly disintegrated after some time, and in all respects resembled those examined previously. In one case the bacteria were washed from the slide and isolated, and their pathogenicity was proved.

*Possible Rôle of the Strands in Epidemiology.* Under the conditions of the current season, there was no natural occurrence of fire blight in the vicinity of Madison, and less than usual in Wisconsin. Consequently, there was little opportunity to gain evidence relating either to the natural occurrence of these strands or their possible rôle in the epidemiology of fire blight. A satisfactory understanding of these matters must, therefore, await further studies. In the meanwhile, attention is invited to the readiness with which the strands may be detached and disseminated by wind, and the possibility that their occurrence may help to explain some of the situations not infrequently encountered by students of fire blight in which the incidence of the disease seems not to accord well with the means hitherto known for its dissemination.



## SUMMARY

A special type of bacterial exudate occurring in the form of hair-like strands on pear, *Pyrus communis*, pedicels, and more rarely on the shoots and fruits, blighted by *Erwinia amylovora* is described. The strands, which were observed on many artificially inoculated blossom clusters in the greenhouse and out of doors, are composed of cells of the fire-blight pathogen bound together by a cementing substance. They are more or less curved, glistening, and usually colorless. Their length varied from a fraction of a millimeter to several centimeters, their width from 8 to 45  $\mu$ . They might be confused with hairs of the host, spider webs, down from cottonwood seeds, etc. However, when placed in a drop of water the strands disintegrate instantly, while all the other similar structures observed retain their form. The strands arise from the internal diseased host tissue, protrude through small openings, and may reach a length of several millimeters in a few hours. They are infective, their bacteria remaining viable for more than seven days. They can easily be broken off from their place of origin and disseminated by the wind. Their possible rôle in the epidemiology of fire blight remains to be investigated.

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# COMMON MOSAIC OF THE GARDEN PEA, *PISUM SATIVUM*<sup>1</sup>

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(Accepted for publication March 9, 1937)

Pea mosaic, a virosis of the garden pea, *Pisum sativum* L., was first reported in the literature by Dickson (2) in 1922. Doolittle and Jones (3), in 1925, reported a pea mosaic disease to be widespread in Wisconsin. Later workers, Osborn (5), Stubbs (8), Zaumeyer and Wade (9), and Pierce (6), on the basis of symptomatology, host range, and physical properties of the viruses, have established the fact that any one of several viruses may cause pea mosaic.

A virus known to be responsible for a mosaic of peas in Idaho was previously described by Pierce (6) as the common pea-mosaic virus (*pea virus 3*) and was considered to be the same virus as that causing the pea mosaic described by Doolittle and Jones (3). Subsequently, Zaumeyer and Wade (9) described a common pea-mosaic virus and a red-clover-mosaic virus, both of which are similar to, if not identical with, *pea virus 3*. Also, Chamberlain (1) has described a pea mosaic in New Zealand, which apparently is identical with our common pea mosaic caused by *pea virus 3*. The relationship of other legume-mosaic viruses to peas has been recently worked out by Pierce (6), and Zaumeyer and Wade (9).

The present paper embodies the results of an extensive investigation of the host range, the transmissibility by insects and through seed, varietal susceptibility, and physical properties of the common pea-mosaic virus (*pea virus 3*). The results presented show that, although *pea virus 3* is not commonly transmitted through the seed, it is transmissible to species of several genera of the family Leguminosae, some of which play an important part in the overwintering of the virus.

## MATERIALS AND METHODS

The common pea-mosaic virus used in this study was the same as that previously (6) identified and named *pea virus 3*. Methods of mechanical inoculation were essentially the same as those described by Rawlins and Tompkins (7). Aerial portions of diseased broad bean plants were ground up in a sterile mortar, and the juice was then strained through cheesecloth in order to remove the gross solid material. Carborundum powder was added directly to the inoculum, and the latter was immediately rubbed over the surface of the leaves by means of a small cheesecloth pad. The leaves were supported by a small card while being inoculated. Aging and thermal inactivation-point studies were made in the usual manner as previously described (6).

<sup>1</sup> Published with the approval of the Director of the Idaho Agricultural Experiment Station as Research Paper No. 158.

Seed supplies for the host range study were obtained from the United States Department of Agriculture, the Idaho Agricultural Experiment Station, and commercial seed companies. As far as could be determined, all species and horticultural varieties were true to name. Seed peas for the tests on seed transmission were harvested from field plots infected with common pea mosaic during the season of 1935.

#### EXPERIMENTAL RESULTS

In order to establish those characters of the common pea-mosaic virus, *pea virus 3*, that are of major importance in identification and control, the symptom expression, host range, varietal susceptibility, seed and insect transmissibility, the thermal death point, and the longevity *in vitro* of the virus were studied.

#### Symptom Expression

The symptoms produced by *pea virus 3* varied considerably, depending upon the species of host plant infected, upon the age of the plants, and upon the environmental conditions. Certain hosts, however, were found to develop specific types of symptoms consistently and may, therefore, be relied upon to aid in the differentiation of *pea virus 3* from other viruses capable of causing mosaic of peas.

The first symptom produced by *pea virus 3* following inoculation to garden pea is a clearing of the veins in the leaves of the new growth (Fig. 1, A). The later symptoms produced by *pea virus 3* are characterized by chlorosis or severe yellowing of the leaves with numerous dark green areas dispersed over the leaflets (Fig. 1, B-F). General stunting of infected plants is a typical characteristic symptom. The symptoms are usually most severe in the upper portions of the plant, and a general chlorosis may or may not be apparent. Certain varieties, such as Alaska and Telephone, tend to show general chlorosis (Fig. 1, B), while others, such as Alderman, Worlds Record, and Market Surprise, tend to show distinct mottling (Fig. 1, C, D, E).

Under greenhouse conditions symptoms of *pea virus 3* infection on blue lupine, *Lupinus angustifolius*, on chick pea, *Cicer arietinum* L., and on *Lathyrus sativus* L. were characterized by necrosis of the tips of the plants and by severe yellowing of the foliage. A complete necrosis of chick peas and blue lupines occurred (Fig. 2, A, B) following inoculation with *pea virus 3*. Symptoms on common vetch, *Vicia sativa* L., were characterized by mottling and severe curling of the leaves.

Pronounced mottling and chlorosis (Fig. 2, C, F) were typical symptoms on broad bean, *Vicia faba* L. and sweet pea, *Lathyrus odoratus* L. Dark green areas were found interspersed in the abnormally yellow-green portions of the leaves. Symptoms on alsike clover, *Trifolium hybridum* L., were a vein clearing and faint mottling (Fig. 2, H). On crimson clover, *T. incar-*

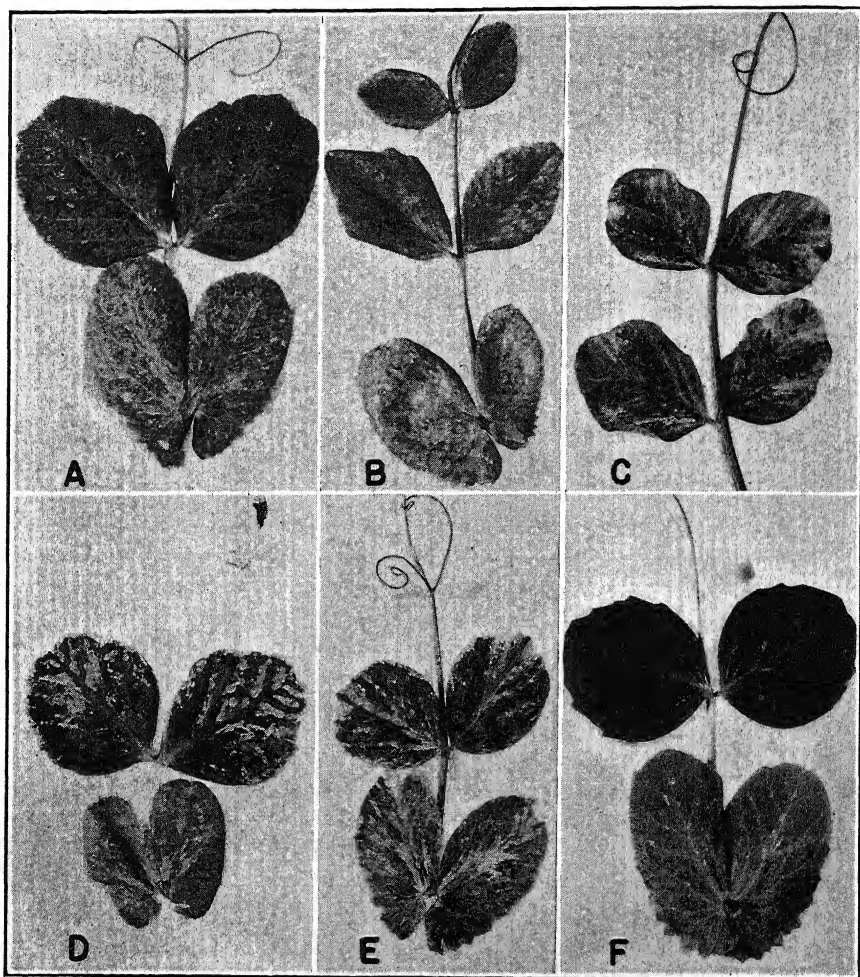


FIG. 1. Leaf and stipule symptoms of the common pea-mosaic virus on certain varieties of peas. A. Prince of Wales, showing vein clearing, an early symptom. B. Telephone, showing mottling and general chlorosis. C, D, and E. Alderman, Worlds Record, and Market Surprise, respectively, showing severe mosaic mottling. F. Market Surprise, noninfected control.

*natum* L. and red clover, *T. pratense* L., the symptoms were a definite yellow mottling (Fig. 2, D, G). The symptoms obtained on garden peas, field peas, sweet peas, blue lupines, white lupines, broad beans, red clover, and alsike clover were practically identical to those obtained by Chamberlain (1) with a pea-mosaic virus in New Zealand. These symptoms also agree closely with those obtained by Zaumeyer and Wade (9) with their common pea-mosaic virus.

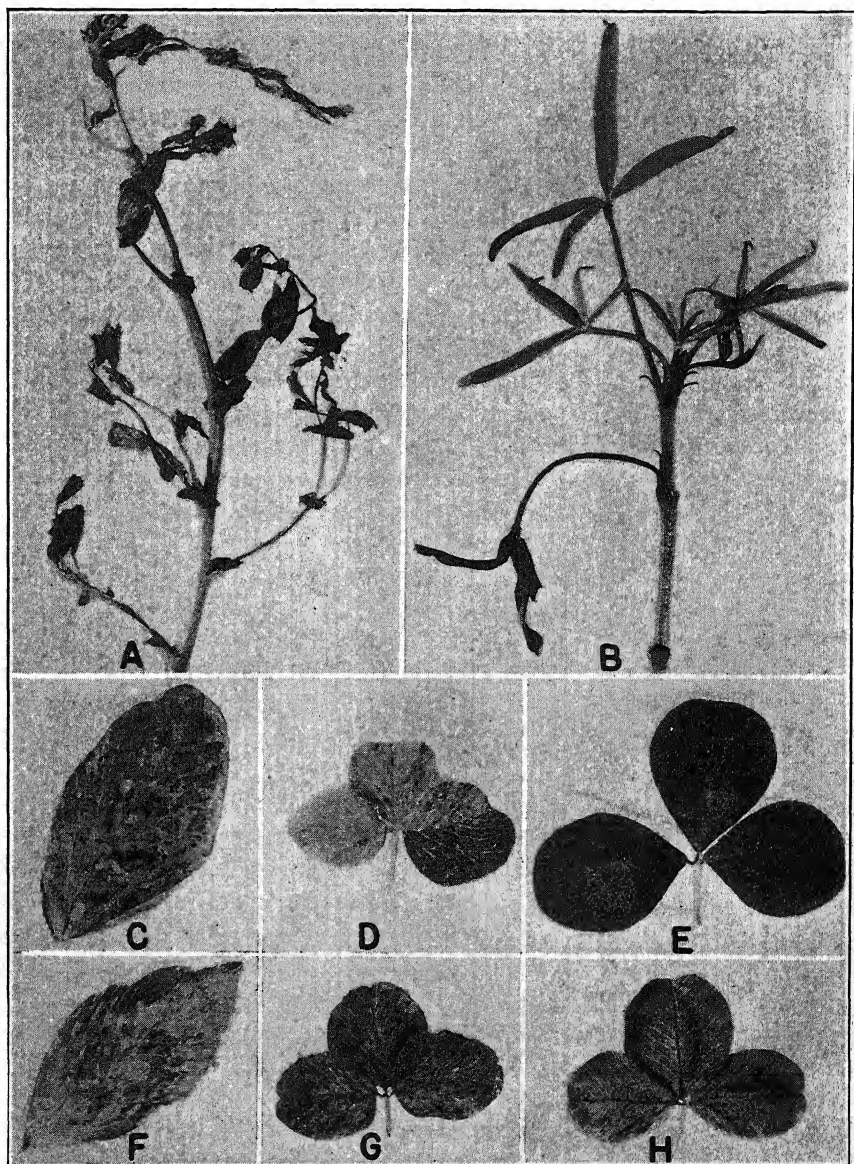


FIG. 2. Symptoms of the common pea-mosaic virus on certain legume species. A. Necrosis and yellowing on *Cicer arietinum*. B. *Lupinus angustifolius* showing necrosis. C. Typical mottling symptoms on broad bean leaf. D. Severe chlorosis and mottling on crimson clover. E. Crimson clover, noninfected control. F. Infected leaf of sweet pea showing mottling. G. Red clover showing mosaic mottling. H. Infected alsike clover showing general chlorosis and vein clearing.

## Host Range

The host-range studies included a total of 2,424 plants representing 32 families, 60 genera, and 94 species. No infection with *pea virus 3* was obtained in any plant family other than the family Leguminosae. The following nonleguminous species were tested and all found to be nonsusceptible: *Thunbergia alata* Bojer., *Mesembryanthemum crystallinum* L., *Impatiens balsamina* L., *Myosotis sylvatica* Hoffm., *Campanula pyramidalis* L., *C. medium* L., *Dianthus barbatus* L., *D. deltoides* L., *Saponaria vaccaria* L., *Calendula officinalis* L., *Aster* spp., *Calonyction aculeatum* H., *Brassica oleracea* var. *capitata* L., *Mathiola incana* R. Br., *Raphanus sativus* L., *Cucurbita maxima* Duches., *Cucumis sativus* L., *Scabiosa atropurpurea* L., *Ricinus communis* L., *Geranium sanguineum* L., *Setaria glauca* (L.) Beauv., *Bromus brizaeformis* F. M., *Phacelia campanularia* Gray., *Allium cepa* L., *Linum grandiflorum* Desf., *Lobelia* sp., *Lavatera trimestris* L., *Mirabilis jalapa* L., *Oenothera missouriensis* S., *Armeria formosa* Hort., *Phlox drummondii* Hook., *Rumex acetosa* L., *Delphinium grandiflorum* L., *Digitalis purpurea* L., *Verbascum thapsus* L., *Petunia hybrida* Vilm., *Lycopersicon esculentum* var. *commune* Bailey, *Nicotiana tabacum* L., *Solanum nigrum* L., *Physalis pubescens* L., *Tropaeolum majus* L., *Pastinaca sativa* L., *Valerianaella locusta* var. *olitoria* L., *Viola tricolor* var. *hortensis* DC.

The tests with members of the legume family included 1,596 plants representing 18 genera, 50 species, and 82 horticultural varieties or strains. Three hundred thirty leguminous plants of 9 genera, 28 species, and 32 horticultural varieties were susceptible to *pea virus 3*. In table 1 is recorded the number of plants inoculated, number infected, and percentage infected of the leguminous plants found susceptible to common pea mosaic. Susceptible host plants included chick pea, *Cicer arietinum* L.; *Desmodium canadense* (L.) DC.; sweet pea, *Lathyrus odoratus* L.; grass pea, *L. sativus* L.; blue lupine, *Lupinus angustifolius*; white lupine, *L. albus* L.; *L. hartwegii* Lindl.; *L. nanus* Dougl.; *L. densiflorus* Benth.; spotted bur clover, *Medicago arabica* Huds.; toothed bur clover, *M. hispida* Gaertn.; hubam clover, *Melilotus alba* var. *annua* Coe.; white sweet clover and alpha white sweet clover, *M. alba* Desr.; annual yellow sweet clover, *M. indica* All.; yellow sweet clover, *M. officinalis* Lam.; tepary bean, *Phaseolus acutifolius* var. *latifolius* Freem.; *Trifolium procumbens* L.; crimson clover, *T. incarnatum* L.; *T. reflexum* L.; *T. dubium* Sibth.; *T. agarium* L.; *T. carolinianum* Michx.; Persian clover, *T. suaveolens*; alsike, *T. hybridum* L.; red clover, *T. pratense* L.; cluster clover, *T. glomeratum* L.; broad bean, *Vicia faba* L.; and common vetch, *V. sativa* L. The infection of the plants reported as susceptible was checked in each case by inoculation of infectious juice to the Alderman variety of peas.

The following species of Leguminosae were tested, but no infection was obtained with *pea virus 3*: *Apios tuberosa* Moench, *Baptisia australis* R. Br.,



TABLE 1.—*Species of the Leguminosae family found susceptible to infection with pea virus 3*

Genus and species	Common name	Plants inoculated	Plants infected	Plants infected
		Num- ber	Num- ber	Per cent
<i>Cicer arietinum</i> L. ....	Chick pea	34	30	88
<i>Desmodium canadense</i> (L.) DC. ....	Trefoil	3	3	100
<i>Lathyrus odoratus</i> L. ....	Sweet pea, Late Spencer	34	20	59
	Late garden flowering	20	15	75
<i>L. sativus</i> L. ....	Grass pea	24	4	17
<i>Lupinus angustifolius</i> ....	Blue lupine	47	42	89
<i>L. albus</i> L. ....	White lupine	22	14	64
<i>L. densiflorus</i> Benth. ....		14	4	29
<i>L. hartwegii</i> Lindl. ....		28	15	54
<i>L. nanus</i> Dougl. ....		8	4	50
<i>Medicago arabica</i> Huds. ....	Spotted bur clover	10	1	10
<i>M. hispida</i> Gaertn. ....	Toothed bur clover	10	4	40
<i>Melilotus alba</i> var. <i>annua</i> Coe ....	Hubam clover	10	3	30
<i>M. alba</i> Desr. ....	White sweet clover	8	3	38
	Alpha white sweet clover	10	5	50
<i>M. indica</i> All. ....	Annual yellow sweet clover	10	6	60
<i>M. officinalis</i> Lam. ....	Yellow sweet clover	9	2	22
<i>Phaseolus acutifolius</i> var. <i>latifolius</i> Freem. ....	Tepary bean	32	2	6
<i>Trifolium glomeratum</i> ....	Cluster clover	10	9	90
<i>T. procumbens</i> L. ....	Low hop clover	4	3	75
<i>T. incarnatum</i> L. ....	Crimson clover	19	11	58
<i>T. reflexum</i> L. ....	Buffalo clover	7	6	86
<i>T. dubium</i> Sibth. ....		7	7	100
<i>T. agrarium</i> L. ....	Hop clover	9	3	33
<i>T. carolinianum</i> Michx. ....		10	8	80
<i>T. suaveolens</i> ....	Persian clover	10	10	100
<i>T. hybridum</i> L. ....	Alsike clover	10	3	30
<i>T. pratense</i> L. ....	Red clover	138	14	10
<i>Vicia faba</i> var. <i>minor</i> ....	Broad bean	60	58	97
<i>V. faba</i> var. <i>major</i> ....	Broad bean	15	15	100
<i>V. sativa</i> L. ....	Common or spring vetch	30	6	20

*Cassia medsgeri* Schafer., *Crotalaria retusa* L., *Dolichos lignosus* L., *D. lab-lab* L., *Glycine max* Merr. (9 varieties), *Lathyrus latifolius* L., *L. tuberosus* L., *Lotus ornithopodioides*, *M. lupulina* L., *M. sativa* L. (11 varieties), *P. aconitifolius* Jacq., *P. aureus* Roxb., *P. coccineus* L., *P. vulgaris* L. (8 varieties), *P. limensis* Macf., *Pueraria hirsuta* Schneid., *Trifolium repens* L., *T. medium* L., *V. villosa* Roth., *Vigna sinensis* Endl.

#### Varietal Susceptibility

A total of 44 varieties of garden peas and 17 varieties of field peas were found susceptible to *pea virus 3* (Table 2). The following varieties were tested and found to be resistant: American Wonder, Cannors Gem, Dwarf White Sugar, Early Bird, Horal, Hundredfold, Laxton's Superb, Little Mar-

vel, Morse Market, Notts Excelsior, Onward, Perfection, Premium Gem, Rice's 13, Surprise, Thomas Laxton, White Marrowfat, Wisconsin Early Sweet, Zwaan's Banquet, Mackay, and Tom Thumb.

TABLE 2.—*Varieties of garden peas, Pisum sativum, and field peas, P. sativum var. arvense, found susceptible to pea virus 3*

Variety	No. samples	Plants inoculated	Plants infected	Plants infected
		Number	Number	Per cent
<i>Pisum sativum</i>				
Advancer .....	1	12	2	17
Alaska .....	7	127	99	78
Aleross .....	1	24	9	37
Alderman .....	6	148	120	81
Ameer Claudit .....	1	10	10	100
Blue Bantam .....	1	13	3	23
British Lion .....	1	10	9	90
Champion of England .....	1	18	10	56
Dark Laxtonian .....	1	9	6	67
Discovery .....	1	20	17	85
Dwarf Alderman .....	2	97	79	81
Dwarf Defiance .....	1	10	8	80
Dwarf Gray Sugar .....	1	30	17	57
Dwarf Telephone .....	2	38	17	44
Everbearing .....	3	70	38	54
Extra Early Pilot .....	1	28	8	29
First and Best .....	1	36	26	72
Giant Stride .....	1	7	6	86
Gradus .....	3	27	15	55
Green Admiral .....	2	28	17	60
Improved Gradus .....	1	10	3	30
Improved Alderman .....	1	10	7	70
Laxtonian .....	1	8	3	38
Laxton's Progress .....	3	48	22	46
Mammoth Melting Sugar .....	1	8	4	50
Market Surprise .....	1	29	27	93
Pilot .....	1	6	2	33
Pioneer .....	2	14	12	85
President Wilson .....	1	7	1	14
Prince of Wales .....	1	29	20	69
Profusion .....	1	12	6	50
Quite Content .....	1	27	19	70
Radio .....	1	34	20	59
Rogers K .....	1	13	10	76
Rogers Winner .....	1	13	12	92
Senator .....	1	20	12	60
Stella or Easy Money .....	1	5	4	80
Stratagem .....	2	29	17	58
Stratah .....	1	10	5	50
Surprise .....	1	26	23	89
Tall Gray Sugar .....	1	13	11	85
Telephone .....	2	46	30	65
Thomas Laxton .....	2	24	13	54
Worlds Record .....	4	40	27	67
Yorkshire Hero .....	1	9	8	89



TABLE 2.—(Continued)

Variety	No. samples	Plants inoculated	Plants infected	Plants infected
		Number	Number	Per cent
<i>Pisum sativum</i> var. <i>arvense</i>				
Arthur .....	1	8	4	50
Australian Winter .....	1	41	22	54
Bangalia .....	1	11	8	73
Blue Prussian .....	1	10	7	70
Canada Field .....	1	26	17	66
Chancellor .....	1	11	7	64
Chang .....	1	13	5	39
Dashaway .....	1	9	3	33
Early Washington .....	1	15	8	53
Hawleys Improved .....	1	13	6	46
Idabells .....	1	21	8	38
New Canadian Beauty .....	1	7	7	100
Ottawa .....	1	14	12	86
Paragon .....	1	16	5	31
Potter .....	1	9	1	11
White Canada .....	1	15	14	93

## Seed Transmission

For the tests on seed transmission, garden-pea seed was collected from plants of Alderman and Dwarf Alderman severely affected with common pea mosaic at Twin Falls, Idaho, in 1935. Seedlings of these varieties were grown in the greenhouse and closely observed for possible cases of seed transmission of mosaic. Three hundred forty-six of these seedlings were grown from seed planted in single plant units. The remainder of the seedlings were grown from bulk lots of seed harvested from plants artificially inoculated early in the season (524 seedlings) and late in the season (157 seedlings), and from plants naturally infected in the field (3,582 seedlings). In all, a total of 4,263 seedlings were grown without a single case of seed-transmitted mosaic. In table 3 a comparison of seed-transmission studies as reported by Dickson (2), Doolittle and Jones (3), Johnson and Jones (4), Pierce (6), Zaumeyer and Wade (9), and Chamberlain (1) is given.

It is apparent from the data presented in table 3 that the common pea-mosaic virus is rarely transmitted through the seed. It is possible that the high percentage of seed transmission reported by Dickson (2) may have been due to the use of a different virus.

## Insect Transmission

Transmission of *pea virus 3* by the pea aphid, *Illinoia pisi* Kalt., has been demonstrated. Aphids, fed on peas infected with *pea virus 3*, were caged on 25 healthy plants of Asgrow 40 peas. Within 3 weeks 16 plants developed typical symptoms of common pea mosaic. Doolittle and Jones (3) and Zaumeyer and Wade (9) also have reported successful transmission by the

pea aphid of the same or a similar virus. Chamberlain (1) obtained successful transmission with *Myzus persicae*, *Macrosiphum gei*, and *Aphis rumicis*.

TABLE 3.—A summary of results of pea mosaic seed-transmission studies as reported by various investigators

Variety	Number of seedlings	Number of seedlings infected	Identity of virus	Authority		
Alderman .....	2888	0	<i>pea virus 3</i>	This paper		
Dwarf Alderman .....	1375	0				
Golden Vine .....	38	29	Unknown	Dickson (2)		
Arthur .....	22	13				
Canadian Beauty .....	24	8				
White Marrowfat .....	17	2				
Chancellor .....	19	2				
Grass Pea .....	20	1				
Alaska .....	1038	0	Probably <i>pea virus 3</i>	Doolittle and Jones (3)		
Ironclad .....	388	0				
Duchess of York .....	493	0				
Prince Edward .....						
Telephone .....						
Carter's Daisy .....						
Sharp's Standard .....						
Eclipse .....						
Unknown .....	12000	rarely	Unknown	Johnson and Jones (4)		
Alderman .....	100	0	Common pea-mosaic virus, probably the same as <i>pea virus 3</i>	Zaumeyer and Wade (9)		
Green Giant .....	2103	8				
Dwarf Telephone .....	185	3				
Potlatch .....	314	0				
Rogers D .....	120	0				
Dwarf Alderman .....	22	0				
Prince of Wales .....	213	0				
Horsford .....	30	1	<i>pea virus 3</i>	Pierce (6)		
Green Feast .....	1400	0	Probably the same as <i>pea virus 3</i>	Chamberlain (1)		
Pioneer .....	65	0				
Great Crop .....	94	0				
Medium Straw Daisy .....	92	0				
Gladiator .....	185	0				
Admiral Beatty .....	119	0				
Pride of the Market .....	182	0				
Prince of Wales .....	495	0				
Partridge .....	50	0				

#### Thermal Death Point

The thermal death point or inactivation point of *pea virus 3* was reported by Pierce (6) to be at 62° to 64° C. for 10 minutes in his determinations made on both peas and broad beans. In table 4 are summarized the results of 3 additional trials on the thermal death point of *pea virus 3* using inoculum from peas and broad beans. A total of 186 seedling garden-pea plants

were inoculated with inoculum subjected to temperatures ranging at 2-degree intervals from 56° to 64° C. A temperature of 60° C. was found sufficient to inactivate *pea virus 3*.

TABLE 4.—Results of thermal death point and longevity *in vitro* determinations of *pea virus 3* as determined by systemic infection on Alderman peas

Thermal death point (10 minutes)			Longevity <i>in vitro</i> at 22° C.		
Temp. ° C.	Plants inoculated	Plants infected	Time aged	Plants inoculated	Plants infected
Inoc.	Number	Number	Days	Number	Number
Control .....	37	19	0	12	8
56 .....	27	4	2	15	6
58 .....	48	2	3	15	0
60 .....	42	0	4	15	0
62 .....	23	0	...	.....	...
64 .....	9	0	...	.....	...

#### Longevity *in Vitro*

Additional data to those previously presented by Pierce (6) on the longevity *in vitro* of *pea virus 3* are given in table 4. No infection was obtained with inoculum aged 3 days or longer at 22° C.

#### DISCUSSION

The evidence presented in this paper shows that the virus of common pea mosaic (*pea virus 3*) can be transmitted to a number of perennial plants belonging to the family Leguminosae. Also, it was shown that the virus is rarely transmitted through the seed. It appears probable, therefore, that certain perennial plants play an important part in overwintering the virus. On the basis of the evidence of Doolittle and Jones (3) that the pea-mosaic virus, which they described as intertransmissible to red clover and not seed-transmitted, it seems reasonable to assume that their virus and *pea virus 3* are very similar if not identical. That other viruses may affect peas has been definitely shown by Osborn (5), Stubbs (8), Pierce (6), and Zaumeyer and Wade (9). The interest now lies in establishing the overwintering hosts for each of the several viruses.

Of the perennial plants found susceptible to *pea virus 3* in this investigation, red clover, alsike clover, and yellow and white sweet clover are economically important forage and seed crops in Idaho. These crops are widely grown and may act as overwintering hosts of *pea virus 3*. Severe pea mosaic infestations in the green-pod pea section of Valley County, Idaho, have been found to be associated with mosaic-infected red clover and alsike clover growing in adjacent fields or along borders and fence rows. Aphids have been associated with the spread of pea mosaic under natural conditions, and it is

probable that aphids carry infection from the overwintering hosts to pea fields in the spring, as suggested by Doolittle and Jones (3). It would appear probable, therefore, that pea growers experiencing trouble with common pea mosaic might obtain some measure of control by isolation of pea plantings from infected perennial clover fields and by cleaning up infected plants along irrigation ditches and fence rows.

One of the most important methods of combatting virus diseases is through the use of resistant varieties. It was found in this investigation that certain pea varieties were resistant from infection with *pea virus 3* when inoculated by the artificial methods used. Some of these resistant varieties, such as Perfection, Cannons' Gem, Horal, and Wisconsin Early Sweet, are widely used in the canning industry and should prove to be very useful as parent stock for the production of new varieties resistant to common pea mosaic.

In addition to suggesting practical measures for the control of common pea mosaic, the data presented will aid materially in differentiating *pea virus 3* from other viruses known to affect peas. With the more general recognition of virus specificity, host range studies have taken on added significance in the matter of virus descriptions. In the present investigations no hosts outside of the family Leguminosae were found for *pea virus 3*. Obviously, then, this virus cannot be confused with the many described viruses affecting plants outside the legume family. The chief problem, therefore, lies in differentiating *pea virus 3* from the other viruses affecting leguminous plants. *Pea virus 3* may be characterized on the basis of symptom expression, non-transmissibility to beans, soybeans, Horal and Perfection peas, and on transmissibility to red clover. Stubbs (8) described a virus (*pea virus 2*) that is similar to *pea virus 3* with the exception that it was nontransmissible to red clover. The enation pea-mosaic virus (*pea virus 1*) of Stubbs (8) and Pierce (6) differs from pea viruses 2 and 3 in being transmissible to Horal and Perfection peas and in producing distinctive foliage enations. The yellow bean-mosaic virus (*bean virus 2*), which Pierce (6) found transmissible to peas, differs from the pea viruses in being transmissible to beans. The red-clover-mosaic virus and the common pea-mosaic virus described by Zaumeyer and Wade (9) appear to be identical with *pea virus 3*. In New Zealand Chamberlain (1) has described a pea mosaic that, on the basis of the symptoms described on differential hosts, appears to be identical to our common pea mosaic caused by *pea virus 3*. The "common mosaic" and "severe mosaic" of peas reported by Johnson and Jones (4) may possibly be caused by *pea virus 1* and *white clover virus 1*, respectively, although there appear to be some points of difference.

It is believed that the data presented on symptomatology, host range, varietal susceptibility, transmission, and physical properties are sufficient to

so characterize *pea virus 3* that it may be readily recognized by other workers.

#### SUMMARY

The symptoms produced by *pea virus 3* on garden peas, *Pisum sativum* L., are characterized by variations ranging from severe yellow mottling and dwarfing to less intense mottling and a general chlorotic condition.

The host range studies of *pea virus 3* included 2,424 annual and perennial plants representing 32 families, 60 genera, and 94 species. Infection was observed on only one family, Leguminosae. Certain species of *Medicago*, *Melilotus*, and *Trifolium* were found to be infected, and it was suggested that certain plants belonging to these species may be possible overwintering hosts.

A total of 62 garden-pea varieties were tested and found to vary in their resistance and susceptibility. Field-pea varieties also varied in their resistance to *pea virus 3*.

A total number of 4,263 seedling garden-pea plants were grown under controlled conditions from seed collected from diseased plants and no evidence of seed transmission of the virus was obtained.

The virus was inactivated by heating at 60° C. for 10 minutes, and by aging *in vitro* for 3 days at 22° C.

It was concluded that the data presented on symptom expression on differential hosts, host range, modes of transmission, and physical properties of the common pea-mosaic virus (*pea virus 3*) were sufficient to differentiate it from other legume viruses.

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# A SIMPLE AND RAPID METHOD FOR IDENTIFYING PLANT VIRUSES IN THE FIELD<sup>1</sup>

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(Received for publication March 16, 1937)

Recent work<sup>2, 3</sup> has shown that the blood test (precipitin test) may be used for identifying and classifying a number of plant viruses. As customarily executed, precipitin testing is a rather exacting procedure and one that requires considerable apparatus, including a constant temperature oven or bath, mechanical shaker, centrifuge, special illuminating chamber, pipettes, and racks. The procedure is also somewhat time-consuming, since the usual technique requires incubating precipitin mixtures in an oven for an hour or more and then allowing the precipitates to settle for 12 to 18 hours before making final readings. The preparation of the materials to be tested may require several days. Under such conditions the blood test, useful though it may be the laboratory and in the hands of a serologist, is out of the question for routine identification of viruses in the field by the non-specialist.

In performing the precipitin test it usually is considered necessary to work with water-clear serum and antigenic solutions. On mixing such solutions the soluble antibody of the serum and the soluble antigen of the virus juice combine to form an insoluble precipitate, which slowly settles out.

In an endeavor to adapt this technique to field use, experiments have been performed to test the possibility of using crude, untreated, expressed plant juices in precipitin testing. Such a procedure was found to be entirely practicable in testing for the presence of 6 different viruses. If virus-immune serum is added to such crude virus-containing juice, a precipitin reaction occurs and quickly becomes evident, since the precipitate that forms involves the suspended plastids and other green material in the juice, and this green matter rapidly settles out. The reaction seems to be of the nature of an agglutination. Microscopic examination of specific serum-virus mixtures shows a clumping of the plastids resembling the agglutination of bacteria or blood corpuscles by specific serum. Non-specific or control mixtures show a uniform distribution of the plastids. It is commonly held by serologists that the precipitin and agglutination reactions are due to the same antibodies, precipitation resulting when the antigenic material is in solution and agglutination when it is in suspension. Occasionally juices are obtained

<sup>1</sup> Published at the expense of The Rockefeller Institute for Medical Research, Princeton, N. J., out of the order determined by the date of receipt of the manuscript. This practice in no wise delays the publication of manuscripts printed at the expense of The American Phytopathological Society or other agency.

<sup>2</sup> Chester, K. S. Serological evidence in the study of the relationships of certain plant viruses. (Abst.) *Phytopath.* 25: 10. 1935.

<sup>3</sup> ———. Serological evidence in plant-virus classification. *Phytopath.* 25: 686-701. 1935.



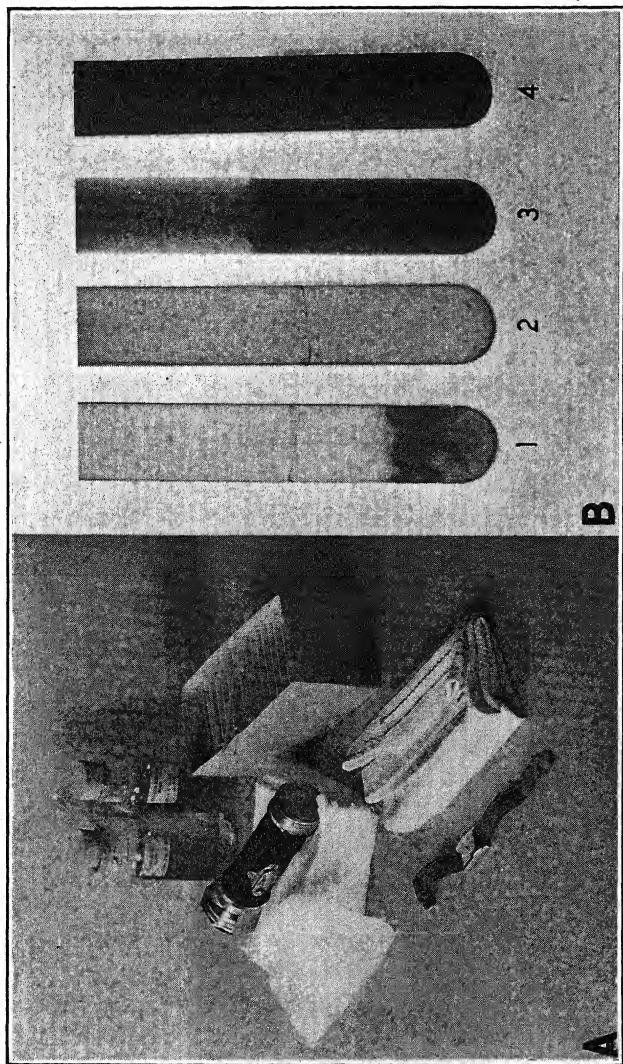


FIG. 1. A: Equipment required in performing 100 blood tests for identifying plant viruses in the field. The number of serum bottles will vary with the object of any given experiment. B: Representative tests ( $\times$  ca.  $2/3$ ). Tube 1—6 drops tobacco-mosaic-diseased tobacco juice + 4.75 cc. absorbed tobacco-mosaic serum at 1:14 dilution. Reaction after  $\frac{1}{2}$  hour. Tube 2—As in tube 1, but using 6 drops healthy tobacco juice. Tube 3—2 cc. latent-potato-mosaic-diseased tobacco juice + 3 cc. absorbed latent-potato-mosaic serum at 1:9. Reaction after 1 hour. Tube 4—As in tube 3 but using 2 cc. healthy tobacco juice.

which settle out spontaneously. This is particularly true of the juices of plants that are old and turning yellow. The nature and elimination of these settlings are being studied.

In performing the simplified field test for viruses, the equipment necessary for 100 tests consists of the following: 100 6-inch squares of gauze or cheese-cloth; 100 5-cc. Wassermann tubes, scratched to indicate the 2-cc. level; 300 cc. of diluted serum (1:9 or higher); a towel; and a flashlight (desirable but optional). This equipment may readily be carried in the pockets or in a small case. If the purpose of the work is to detect a single virus wherever it may occur, only one serum is needed, while if the object is to identify various viruses as they may be found, several sera may be used. The field equipment is illustrated in figure 1, A.

The procedure of testing is as follows: A piece of leaf tissue weighing a few grams (*e.g.*,  $\frac{1}{4}$  to  $\frac{1}{2}$  of a mature tobacco leaf) is wadded into a ball and a square of cheese-cloth drawn about it. In detaching the leaf it should be snapped off at the base or torn in such a way as to avoid infecting the plant with any virus that may contaminate the fingers. The ball of tissue is then pressed and worked with the fingers until it is well crushed. Enough juice is squeezed out to fill a Wassermann tube up to the 2-cc. level; the tube is then filled nearly to the top with serum dilution, shaken, and set in the ground near the test plant. The reaction is observed after a short interval of time. A positive reaction is indicated by the presence of a flaky green precipitate which is best viewed by transmitted light. This rapidly settles to form a dense green deposit about 1 centimeter in depth, overlaid by a paler supernatant fluid. The settled deposit may appear more distinct when viewed by reflected light than when viewed by transmitted light, so both should be employed in making observations. The flashlight is an aid in viewing the tubes by transmitted light. After the test has been made, the hands should be wiped on a towel; washing is unnecessary.

The appearance of the reactions is shown in the accompanying figure (Fig. 1, B).

The strongest reactions first appear after 2-5 min. In no case has a specific reaction appeared after 1 hour. It is necessary to read the tubes at the end of an hour, and preferable to read them twice, once after 15 or 20 min. and a second time after an hour. This may be easily arranged in field work by making a series of tests and then retracing one's steps and reading the tests after the proper lapse of time. The time of mixing may be written on each tube with a wax pencil.

The sera are prepared according to the methods described in an earlier paper.<sup>4</sup> Each serum is absorbed with 2 parts of the juice of healthy specimens of the diseased species used in animal inoculations, and then further diluted to a concentration giving an optimal reaction in laboratory titration.

<sup>4</sup> See footnote 3.



If crude, fresh juice was used in the animal inoculation, the same type of juice must be used for absorption, in order to avoid reactions with healthy plant juices. The serum need not be water-clear. If the sera are to be kept for long intervals at room temperature, they may be preserved by adding phenol to a concentration of .5 per cent. The laboratory titration of a given serum suffices to control the potency of that serum for many months, since the sera lose little of their potency on long-continued storage in frozen condition. In titrating the sera, various amounts of virus juice and serum are mixed, and the proportions giving the best reaction are chosen for future tests. The amount of serum required may be controlled by adjusting its dilution at constant volume (3 cc.), while the optimal amount of plant juice to use with a given serum, should it differ from 2 cc., may be indicated on the bottle containing that serum. Two cc. of virus-containing juice proved satisfactory with all the viruses tested in this study, with the exception of tobacco-mosaic virus. In this case, 5 or 6 drops of virus juice per tube gave the best reaction. It is advisable to test preserved sera against known viruses and healthy plants from time to time. If the plant species to be tested differs from that used in inoculating the animals, the juice of healthy plants of the species to be tested must be used in absorption of the sera.

Table 1 gives the results of testing fresh juices containing a number of different plant viruses.

The method is rapid, as may be seen from the table. The performance of a test requires about a minute, and definite readings may often be made after 2, 5, or 10 minutes. In all cases the reaction is definite after an hour.

TABLE 1.—*Results obtained from testing fresh juices containing a number of different plant viruses*

Crude expressed juice			Homologous serum		Reading	
Virus	Host	Optimal amount	Amount	Optimal <sup>a</sup> dilution	Test apparent after	Test strong after
Latent potato mosaic	Tobacco	2 cc.	3 cc.	1: 9	2-5'	15'
Potato vein-banding	"	2 cc.	3 cc.	1: 9	20'	30'
Tobacco ring-spot	"	2 cc.	3 cc.	1: 9	40'	60'
Etch	"	2 cc.	3 cc.	1: 9	5'	20'
Potato aucuba mosaic	Nicotiana glutinosa	2 cc.	3 cc.	1: 9	10'	30'
Tobacco mosaic	Tobacco	5 drops (= .25 cc.)	4.75 cc.	1: 14	2'	10'
None	Tobacco or Nicotiana glutinosa	.25 cc. — 2 cc.	3-4.75 cc. (all sera)	1: 9 or 1: 14	∞	∞

<sup>a</sup> The process of absorption of sera produces an absorbed serum at a dilution of 1: 3. Such a serum is again diluted 1: 3 in order to give the 1: 9 dilution mentioned in the table. The diluent in each case is .85 per cent NaCl solution.

The reaction is specific for each virus, regardless of host plant, and the juice of healthy plants reacts with none of the sera. Every virus that has been found to give a precipitin reaction according to the customary laboratory technique reacted by the field method. Masking of symptoms does not interfere with the reaction. If a plant is diseased by two different viruses, each component may be identified by its proper serum. A mixed serum may be used whereby any one of several viruses will react with the single serum complex. Accurate readings have been repeatedly made by untrained assistants, and very little instruction was found necessary. Less training is required than for soil pH determinations in the field.

The method appears to be sufficiently inexpensive to warrant its general use. When sheep were used, the initial cost and board per animal amounted to about \$2.00 after credit had been allowed for meat and wool. Each animal yields upwards of a liter of serum, and no more than  $\frac{1}{3}$  cc. is used per test. It is thus possible to perform the tests at a raw-material cost of about 7c per 100 tests for serum.

Although the method of precipitin testing with crude expressed plant juices has been referred to throughout this paper as a field method, it also has demonstrated its usefulness in laboratory research. The use of crude juices has a number of advantages over the older method of using cleared juices. In addition to its rapidity, it offers an opportunity to test virus juices in as unaltered a state as possible, and since a number of viruses are soon inactivated *in vitro*, it may well be that the list of viruses susceptible to the serological methods may be considerably extended through the use of crude juices as test antigens. Preliminary tests indicate that this is the case. For example, using the ordinary precipitin techniques, etch virus juice may be used as both immunization antigen and test antigen, but the two etch strains, severe etch and Blakeslee's Z-mosaic of *Datura*, fail to react as test antigens, although they may be used to prepare sera that react with etch juice. Using the field method, however, both severe etch and Z-mosaic gave good precipitin tests with etch serum. It appears from this that the field technique is more sensitive than the older technique of precipitin testing. If the method described above continues to show a sustained accuracy and sensitivity in comparison with the use of cleared juices, its other advantages warrant its acceptance as the basic method for the serological identification of plant viruses.

Hitherto the diagnosis of plant viruses in the field has depended on observation of symptoms. Conclusive evidence of the identity of a virus could be gained only by plant inoculations involving weeks or months of delay. The symptoms of viruses are often misleading, since two different viruses may cause indistinguishable symptoms on a given host species, while two strains of the same virus may cause very different symptoms on the same host species. While the method described above has been restricted thus far to various

strains of 6 viruses in tobacco, it is hoped that its application on a broader scale may reveal numerous other viruses amenable to the technique, and serve as a guide to roguing, an adjunct or substitute for tuber-indexing, and a tool for the field study of plant-virus occurrence and control.

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## ROSETTE OR LITTLE LEAF OF FRUIT TREES

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The two papers by Kozlowski (8, 9) and the intervening commentary by Chandler and Hoagland (2), which have appeared in this Journal, are worthy of some discussion. Kozlowski (9) deplors the absence of facts from the note of Chandler and Hoagland and professes ignorance of the facts to which these authors refer. In the same paper, however, he cites two of the papers (3, 4, 5, 6, 7) in which these facts have been presented, but, so doing, does not seem to discriminate between fact and hypothesis.

One of the most consistent facts that has emerged from this complex problem is that of complete recovery of affected trees treated by the introduction of zinc, in any one of several forms, into the trunks or main branches. This occurs in the absence of any direct treatment of the soil or the small branches. That the explanation of this frequently demonstrated fact may be a simple zinc deficiency in the tree is a hypothesis and is seemingly so regarded by most workers.

It is probable that certain soil microorganisms are in some way related to the disease (1, 4)<sup>1</sup>. Whether Kozlowski had any of these organisms in culture seems impossible to determine. The nature of the relation between these organisms and the disease is at present obscure.

It is of interest to examine some of the statements made by Kozlowski as representing established facts. Referring to little-leaf trees, it is stated (8) that "... there is no doubt that *Monilia* causes a fatal injury of the trees concerned." This statement is apparently based on the isolation of *Monilia* from affected trees and the production by this fungus of twig lesions on peach trees inoculated in the greenhouse. These lesions were not unlike those commonly produced on peach twigs by the brown rot fungi *Sclerotinia* (*Monilia*) *fructicola* (Winter) Rehm and *Sclerotinia laxa* Aderh. and Ruh. In the peach orchard at Delhi, Merced County, with which the author implies familiarity, twig lesions are sometimes rather numerous, but the great major-

<sup>1</sup> Ark has additional evidence in preparation for publication indicating not only toxic action of bacteria found in the root zone but the prevention of this action by the presence of zinc.

ity of them are of the peach blight caused by *Coryneum beijerinckii* Oud. The Sonoma County orchard referred to by the author is an apple orchard. Branches of rosette trees that die back in this orchard and in other declining orchards of the district are invaded by various fungi, among which *Polystictus versicolor* (L.) Fr. and *Schizophyllum commune* Fr. seem most often to aggravate the injury. In earlier stages, no one organism is consistently found in cultures from such trees. In still earlier stages it is easily possible to recognize leaf and shoot symptoms that involve no necrosis whatever.

Again, it is stated (9) that "These circumstances explain why the rosette of various fruit trees is limited in the United States to certain warm, highly humid regions or, to be more specific, why it is rather common in the interior valleys of California, and does not occur east of the Rocky Mountains." The designation of the San Joaquin Valley as a humid area is a novelty which does not require discussion.

That "soil conditions" play a part in the production of the disease does not constitute a departure from prevalent opinion. The nature of this function is, however, still largely a matter of speculation.<sup>2</sup> It is possible that obstructions to free drainage are involved, not through loss of nitrogen, as the author concludes (9), but by favoring organisms (1) that are directly or indirectly injurious to plants. It is true that the disease often is found in orchards with a low level of nutrients in the soil. It is equally true, however, that in some areas the disease occurs only on land that has been used for long periods as stock corrals. Furthermore in many field experiments heavy applications of nitrogen, phosphorus, potassium, calcium, magnesium, and sulphur have been made without benefit to the affected trees (3). The disease has appeared in severe form under greenhouse conditions when plants were grown in subsoil from affected orchards, notwithstanding the addition of calcium nitrate, potassium phosphate, and magnesium sulphate (7). It is probably true that applications of zinc sulphate to the soil does, as stated (9), tend to release some phosphorus and potassium, but it is not readily conceivable that the equally curative applications of zinc to the tops of trees appreciably alters the nutrients in the soil.

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<sup>2</sup> It may be worthy of mention that the chemists who made the analyses represented in Mr. Kozlowski's latest paper (9) did not participate in the inferences and conclusions drawn from the results.

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## A FOLIAGE YELLOWING AND FLORAL INJURY OF ALFALFA ASSOCIATED WITH HEAT AND DROUGHT<sup>1</sup>

FRED RUEEL JONES

(Accepted for publication March 19, 1937)

Two pathological conditions of alfalfa, apparently undescribed, though probably not unusual, became conspicuous at Madison, Wisconsin, during the unusual heat and drought of the summer of 1936. The first is a yellowing and drying of the tops of individual plants or groups of plants; the second is an extensive dying of buds found associated with restricted terminal growth during the hottest part of the summer.

Yellowing was noted in the first crop on June 18, shortly before cutting in fields where drought appeared to be checking growth. Individual plants or sometimes groups of plants showed in varying degree a yellowing of the upper foliage, and, in extreme cases, a dried chaffy appearance of the terminal undeveloped leaves and flower buds. The yellowing often began abruptly with the foliage at a node in the upper third of the stem, or, when the main stem was branched, at a corresponding node on the several branches. Yellowed plants had apparently ceased growth at least a week prior to observation, since they usually bore only buds, while surrounding plants were in blossom. In spite of this checked growth, the yellow plants were often as tall as those about them, suggesting that, originally, they had been the most vigorous in the field. The distribution and appearance of these yellowed plants was very different from that of plants yellowed by infestation with the potato leaf hopper, very few of which were found in the fields where this condition was most conspicuous.

A yellowing of the same character was even more abundant and conspicuous in the second crop about July 15. The fields under observation had been cut about June 20, and were poor and uneven from drought. As a rule,

<sup>1</sup> Alfalfa disease investigations of the Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, in cooperation with the Wisconsin Agricultural Experiment Station.

affected plants had larger stems and appeared to have recovered from cutting with a more succulent growth than had their dark green neighbors. The yellowing did not spread progressively through the field. It seemed probable that the yellow and the green plants showed strikingly contrasted effects of drought. The yellow plants may have been those that, after cutting, still had access to a little water and started vigorous growth with large leaves, but, when the water was largely exhausted, stopped growth and turned yellow. The green plants, on the other hand, may have been restricted to soil so dry that growth was slower with smaller leaves, which soon assumed the dark green so characteristic of fields in need of irrigation. Crowns and roots of the yellowed plants appeared normal and no evidence of permanent damage was found.

The death of buds during the heat and drought of summer was most conspicuous about July 19 in fields where yellowed plants appeared as previously noted. The mean daily temperature of the 10 days preceding this date had been 87.2° F., and the maximum 107° F. on the 14th. In the better parts of the field the stems were 10 to 15 inches tall, having 10 to 12 well developed internodes and several very short internodes at the top. At the topmost nodes between the short internodes and in terminal position, bud clusters were usually found, but the rachises usually had failed to elongate, leaving the buds closely compacted. Thus the stems appeared to be surmounted by groups of bud clusters resembling tiny more or less perfectly formed clover heads before the flowers are open. Many of the bud clusters were dead and bleached, but they remained in place for days surrounded by the small dark green leaves standing nearly upright from the nodes below in a small rosette. The fields in which this condition was general had a strikingly characteristic appearance.

The dying of the buds seemed to be associated with a cessation of the terminal growth of the plants on which it occurred. Thus, if heat and drought caused the destruction of buds, it may not have been through a selective action upon those buds, but in consequence of the arrested growth in the region as a whole where even the leaves remained exceedingly small.

Somewhat later these plants did blossom and set some seed. On July 17 and 18 about  $\frac{3}{4}$  inch of rain fell, and the mean daily temperature for the 10 days following the 19th was 12 degrees lower than for the 10 preceding days. Unfortunately, consecutive detailed observations were not taken of the growth of the plants during the transition from the period in which buds mostly died to that in which blossoms developed freely. However, it appears that heat and drought, conditions usually considered favorable for seed setting, in this case, while most severe, exceeded their favorable range with consequences as described above.



## PHYTOPATHOLOGICAL NOTES

*Chlorosis of Citrus in Puerto Rico.*—Citrus leaf chloroses due to plant nutrient deficiencies have been reported in almost every region in which citrus plants are grown. Information regarding the causes and distribution of these diseases is in many cases incomplete. This note describes the symptoms and soil relations of a chlorosis causing losses to grapefruit growers in Puerto Rico, and reports the beneficial effects on diseased trees of zinc sulphate, already used elsewhere successfully in the treatment of "mottle-leaf" in citrus. It thus records the occurrence in an additional geographical area of what appears to be a similar deficiency disease.

The symptoms on diseased grapefruit trees in Puerto Rico resemble closely those described for the citrus disease known as "mottle-leaf" in California and "frenching" in Florida. Irregular chlorotic blotches first develop between the larger secondary veins on each side of the leaf midrib, becoming more pronouncedly yellow and increasing in area with increased severity of the disease. In severe cases tissues next to the larger veins and midrib remain green, while the rest of the leaf becomes completely yellow. Trees affected for several seasons frequently produce multiple buds resulting in a bushy growth at the ends of part or most of the branches. Severely affected trees bear little or no fruit and finally become so weak that they are commercially valueless.

Diseased trees in Puerto Rico have been found on areas of alkaline soil of sedimentary origin, testing pH 8.0 to 8.5 near affected trees, none having yet been found on the acid soils of the island.

In preliminary tests, lots of 5-year-old, severely diseased trees were sprayed with water solutions of copper sulphate, iron sulphate, zinc sulphate, and manganese sulphate. Three weeks after the sprays were applied trees treated with zinc sulphate began to show signs of response. New healthy appearing leaves were formed, and some of the chlorotic leaves began to recover their green color. Five weeks and 7 weeks after treatment diseased trees sprayed with zinc sulphate continued to show favorable recovery. Trees treated with copper sulphate shed their leaves. Iron sulphate and manganese sulphate gave no response.—JAMES H. JENSEN, Puerto Rico Experiment Station, Office of Experiment Stations, U. S. Department of Agriculture, Mayaguez, Puerto Rico.

*Sunscald of Tulip Flowers.*—This injury, a manifest drying and shriveling of perianth segments along their upper edges, was noticed on May 10, 1936, among a display planting of thousands of tulips in beds bordering a macadam walk at the Brooklyn Botanic Garden. Flowers of many colors and

types were affected—Darwin, Cottage, Breeder, Double, Lily-flowered, and Parrot. But the extent of the injury was not uniform throughout, and about half of the 50 varieties showed no injury.<sup>1</sup>

The trouble evidently resulted from the extremely hot weather and intense sunlight of May 8 and 9, 1936, following a week of weather favorable to very rapid growth. On May 8 and 9 the maximum temperatures in the shade recorded at the New York (Manhattan) station of the U. S. Weather Bureau were 87° and 90° F., respectively. On the 9th the thermometer (partially shaded) at the Brooklyn Botanic Garden registered 106°: it is probable that temperatures of 120° to 130° were reached in full sunlight. The effect doubtless was intensified by the heat reflected from the pavement bordering the planting of tulips.

Definite evidence of the cause of the injury was presented by the fact that plants of several varieties, *e.g.*, The Bishop and Valentine, happened to be so situated that some were partly in the shade of a tree and others were in full sunlight: those flowers in full sunlight were badly affected, while those in the shade were entirely uninjured (Fig 1, A and B).

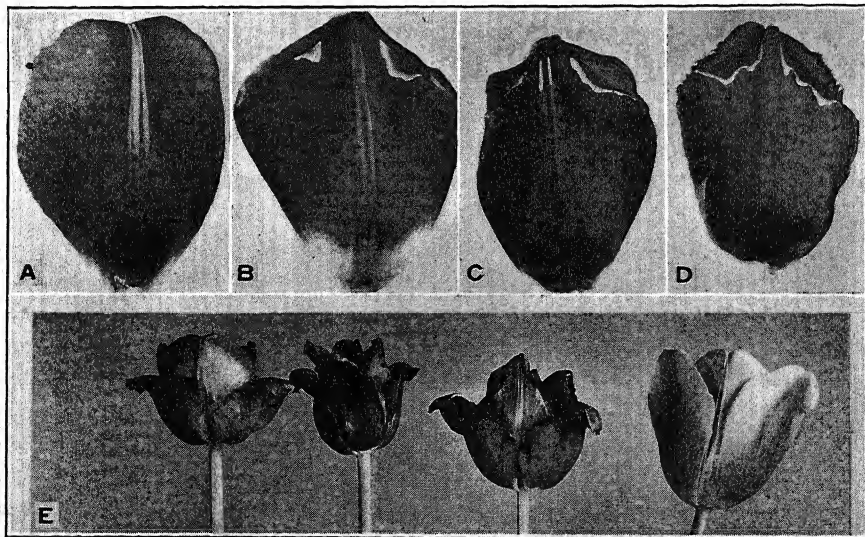


FIG. 1. A-D. Perianth segments of tulip flowers in sun and shade, May 10, 1936. A and B. The Bishop. A, from flower in shade; B, from flower in sun. C and D. Farncombe Sanders and Sundew, in sun. E. Left to right: The Bishop, in shade; Indian Chief, in sun; The Bishop, in sun; Princess Mary, in sun.

<sup>1</sup> The truth of the following statement (from Sorauer's Manual of Plant Diseases, 3rd ed. Trans. by Frances Dorrance, 1914, p. 638) was well borne out: "Not only has each species its special requirements as to the amount of heat which it can endure, but even within a wide range of heat, the different individuals in each species, and indeed the different developmental stages, behave quite differently."



In general, the burned areas showed a fairly uniform pattern, extending some distance down the perianth segment in the thinner tissue at the sides, but leaving intact the thicker, central portion where the principal veins run out almost to the tip, giving an angular outline to the uninjured part of each segment (Fig. 1, B-D). This peculiar configuration furnishes secondary evidence that the injury was sunscald.

The peculiar outline just referred to may be interpreted as due to the fact that the larger veins were able to supply sufficient water to their immediately contiguous areas, while in parts more remote from them the rate of water supply was inadequate to replace the rapid loss by transpiration. There is, however, no reason to assume, in this case, that either the root system or the available soil moisture was inadequate. The condition is to be explained, rather, as a direct effect of heat, causing the death (perhaps from coagulation) of the living protoplasm when the latter is subjected to too high temperatures. And, of course, the most susceptible areas would be the thin, delicate tissues near the edges of the perianth segments.

How much of the injury was due to the excessive heat of the atmosphere, and how much to the direct effect of the intense sunlight, it is, of course, impossible to judge.<sup>2</sup> It is clear, however, that a combination of the two was often fatal to some of the tissues, while the heat of the atmosphere was, by itself, insufficient to cause injury, as is shown by the fact that the flowers in the shade remained intact. We must not overlook the fact, however, that sunlight, both direct and reflected, carries heat waves with it, so that the plants in the open were exposed to much greater heat than those in the shade, even though the air temperature probably would vary little under the two conditions.

On the same day another case of sunscald was reported from Babylon, Long Island, N. Y., where the young leaves of a red-leaved Japanese maple, *Acer palmatum* var., were badly burned; and the injured areas of the leaf lobes were somewhat similar in configuration to those of the tulip flower segments. During a trip through southern Connecticut at about this time, the writer observed sunscald of tulips also near Norwalk and in Bridgeport.

It is of interest to note that many of the tulips in the Brooklyn Botanic Garden collection were more or less immune from the trouble, either because of tissue structure or perhaps also because of inherent protoplasmic resistance. In tomato fruits, Harvey<sup>3</sup> finds that red fruits are injured (by sunscald) less than green fruits. The reason for this, he says, is that the red light, reflected from the red fruit, contains the greater part of the energy of

<sup>2</sup> Wartenberg, H. Kälte und Hitze als Todesursache der Pflanze und als Ursache der Pflanzenkrankheiten. p. 578. In P. Sorauer's Handbuch der Pflanzenkrankheiten. Edited by Dr. O. Appel. Sixth ed. Erster Band. Die nichtparasitären und Virus-Krankheiten. Erster Teil. Berlin. 1933.

<sup>3</sup> Harvey, R. B. Conditions for heat canker and sunscald in plants. Jour. Forestry 23: 392-394. 1925.

sunlight. In our tulip flowers we found no relation of color to degree of injury.

Lists of those varieties injured and those uninjured, even though in full sunlight, follow. Where no other notation is made, flowers were exposed to full sunlight:

<i>Injured</i>	<i>Uninjured</i>
Almirante (Lily-fld.), slight injury	Almirante (Lily-fld.)
Anne Mary (Rembrandt), some individuals <sup>4</sup>	Ambrosia (Cottage)
Aphrodite, slight injury	Anton Mauve (Darwin)
Arethusa (Cottage)	Avis Kennicott (Cottage)
Baronne de la Tonnaye (Darwin), slight injury	Carrara (Cottage)
Bartigon (Darwin)	Cherbourg (Breeder)
Blue Flag (Double late)	Dillenburg (Breeder), in both sun and shade
Dido (Cottage), slight injury	Eclipse (Darwin), in shade
Eclipse (Darwin)	Fantasy (Parrot)
Farncombe Sanders	Golden Beauty (Darwin)
Faust (Darwin)	Jeanne Desor (Cottage)
Gen. Pershing	La Fiancée (Breeder)
Giant (Darwin)	Louis XIV (Breeder)
Indian Chief (Breeder)	Lucifer (Breeder), in shade
John Ruskin (Cottage), some individuals	Marcellina (Lily-fld.)
King George V, slight injury	Marechal Victor (Breeder)
Leda (Cottage)	Melicette (Darwin)
Martha (Lily-fld.)	Mrs. Moon (Cottage)
Mayflower (Cottage), rather old	Persimmon
Monica (Darwin)	Princess Mary (Darwin)
Penserosa (Double late)	Rosabella (Cottage)
Prince of Wales (Darwin)	The Bishop (Darwin), in shade
Princess Elizabeth (Darwin)	Tricolor (Darwin)
Sundew (Parrot)	Valentine (Darwin), in shade
The Bishop (Darwin)	White Queen (Darwin)
Valentine (Darwin)	Yellow Giant (Darwin)
Venus (Darwin)	
Zwanenburg (Darwin)	

As a remedy for this trouble, it is obvious that during unwonted hot periods blossoming tulips should be shaded.—ARTHUR H. GRAVES, Brooklyn Botanic Garden, Brooklyn, N. Y.

*New Hosts and Distribution of Rehmiellopsis bohémica.*—In 1933 the occurrence of a needle and twig blight of *Abies concolor* Lindl. and Gord., caused by *Rehmiellopsis bohémica* Bub. and Kab. (*R. abietis* Rostr.<sup>5</sup>), was reported<sup>6</sup> for eastern Massachusetts, Maine, and New York. Since that time

<sup>4</sup> The age of the flower is an important factor that should not be overlooked. See footnote 1.

<sup>5</sup> It has been recently called to the author's attention by G. D. Darker of the Farlow Herbarium, Harvard University, that a fungus, originally described in 1902 by Emil Rostrup as *Sphaerella abietis* (*His Plantepatologi*, 640 pp. København. 1902. see p. 597), was considered synonymous with *Rehmiellopsis bohémica* Bub. and Kab. by Ove Rostrup in 1916 (see Footnote 12), who made of it a new combination, *R. abietis*. The fungus *S. abietis* Rostr. was placed in the genus *Mycosphaerella* by Lindau (Lindau, G. Die pflanzlichen Parasiten. In Sorauer, P. Handbuch der Pflanzenkrankheiten. Ed. 3. Band 2. Paul Parey, Berlin. 1905-1908. see p. 534). A further study of type material will be necessary to determine the correct combination.

<sup>6</sup> Waterman, Alma M., and M. A. McKenzie. A disease of Colorado fir. *Phytopath.* 23: 108-109. 1933.

the disease has been found in Rhode Island on a few trees of *A. concolor* and one of *A. cephalonica* Loud.; in Edgewood, British Columbia,<sup>7</sup> fairly common on *A. lasiocarpa* Nutt.; and in Maine in the summer of 1935 on *A. balsamea* Mill., collected by J. R. Hansbrough of the Division of Forest Pathology, Bureau of Plant Industry, U.S.D.A., from a single tree in the natural growth along a roadside in Eustis, Maine, near the Canadian border. In the summer of 1936, a careful inspection of the balsam firs in this region showed the disease to be fairly well distributed in Jim Pond Township, Eustis, and Stratton, Maine, and particularly abundant on the young seedling trees in the valley just north of Mt. Bigelow. The localities in Maine where the disease had previously been collected on *A. concolor* were situated in the southern part of the State, around Augusta and Portland.

*Abies lasiocarpa* and *A. balsamea* have not previously been reported as hosts for this fungus, although *A. arizonica* Merr. (*A. lasiocarpa* var. *arizonica* Lamm.) was reported in Denmark<sup>8</sup> (see p. 204) as a host for *Mycosphaerella abietis* (Rostr.) Lind.<sup>2</sup> Reports of the occurrence of the fungus on *A. cephalonica* have been made from Denmark<sup>9</sup> and from Scotland,<sup>10</sup> but it has not previously been reported on this host in the United States.

In the fall of 1933, a total of 26 small nursery trees of 7 species were planted among the most severely diseased trees in eastern Massachusetts to determine whether these species might be susceptible to natural infection under the conditions existing in this plantation.<sup>11</sup> The respective number of trees of each species was as follows: 4 each of *Abies nobilis* Lindl., *A. fraseri* Poir., *A. alba* Mill., *A. arizonica* Merr., *A. veitchii* Lindl., and *A. homolepis* Sieb. and Zucc.; and 2 of *A. holophylla* Maxim. The trees were so distributed among the diseased trees that all would be equally exposed to infection. In October, 1935, an inspection of the trees showed the following results: *A. nobilis*—all 4 infected; *A. fraseri*—3 infected, 1 dead (probably from winter injury); *A. alba*—2 unhealthy but not infected, 2 dead from winter injury; *A. arizonica*—1 unhealthy but not infected, 3 dead from winter injury; *A. veitchii*—2 dead from winter injury, 2 healthy; *A. homolepis*—all 4 healthy; *A. holophylla*—2 healthy.

*Abies nobilis* has been reported as a host for the fungus in Denmark<sup>12</sup> and

<sup>7</sup> Collections in British Columbia were made in 1932 by L. N. Goodding, formerly of the Division of Blister Rust Control, U.S.D.A., and in 1935 by J. W. Kimmey of the Division of Forest Pathology, Bureau of Plant Industry, U.S.D.A.

<sup>8</sup> Lind, J. Danish fungi as represented in the herbarium of E. Rostrup. 648 pp. Gyldendalske Boghandel, Copenhagen. 1913.

<sup>9</sup> Rostrup, E. En Sygdom hos Aedelgran, foraarsaget af *Sphaerella abietis*. Tidsskr. Skovvaesen 17A: 37-41. 1905.

<sup>10</sup> Wilson, M., and J. Macdonald. A new disease of the silver firs in Scotland. Roy. Scot. Arbor. Soc. Trans. 38: 114-118. 1924.

<sup>11</sup> This experiment was begun by M. A. McKenzie, formerly Agent in the Division of Forest Pathology.

<sup>12</sup> Rostrup, O. Bidrag til Danmarks svampeflora. I. Dansk. Bot. Arkiv. v. 2, no. 5. 1916.

in Scotland,<sup>13</sup> but *A. fraseri* has not previously been reported. *Abies homolepis*, *A. holophylla*, and *A. veitchii* are apparently resistant to infection under the conditions in this particular plantation.—ALMA M. WATERMAN, Division of Forest Pathology, Bureau of Plant Industry, in cooperation with the Osborn Botanical Laboratory, Yale University, New Haven, Conn.

<sup>13</sup> See footnote 10.

## BOOK REVIEW

S. N. Das Gupta. *Saltation in Fungi*. 83 pages. Lucknow University Studies No. V. Newul Kishore Press, Lucknow, India, 1936. Bibliography: pp. 66–83.

This booklet is based on a course of three lectures delivered by the author at the University of Lucknow. Students of fungi will find it very useful because it brings together many data scattered throughout the literature and gives a comprehensive view of the interesting phenomenon known as saltation. Dr. Gupta does not attempt to analyze the fundamental factors responsible for saltation because he realizes that our knowledge concerning this phenomenon is still meager. However, he believes that a better understanding may ensue from the nuclear history of fungi.

The booklet is divided into six chapters and a bibliography. The first chapter is an introduction to the phenomenon of saltation. The second chapter is termed “genetical” and is devoted to theories and explanations offered by different workers. The author concludes that anastomoses of hyphae, while affecting “heterocaryosis,” may not produce myxochimaeras, and that many of the saltations are mere segregations of hybrid characters, although, when a fungus gives rise to a large number of saltants, the possibility of its being a hybrid strain correspondingly decreases. The third chapter classifies the types of saltation as follows: saltation in spores; sectorial and masked saltation in mycelium; orthogenetic and cyclic saltations; saltation into complementary strains; saltation with age; ever-saltating and reversing phenomena. The fourth chapter is devoted to induced saltation and summarizes the effect of chemicals, hydrogen-ion concentration, oxidation, wounding, temperature, light, and radiation. The fifth chapter discusses the differences that may be observed between the parent and the saltant, such as macroscopic variation, zonation, reproduction, microscopic appearance of hyphae, cells, and spores, the physiological behavior, and pathogenicity. The sixth chapter is a short one and is devoted to saltation, bud variation, and plant chimaeras in higher plants.

After reading the booklet one immediately wishes for a more exhaustive text on the subject. As the author himself points out, the work is by no means complete; a number of pertinent publications have been omitted, and no mention is made of saltation (dissociation) in bacteria. This is a vast and important field in which many exhaustive and interesting studies have been made. Dr. Gupta's booklet should be the forerunner of a more inclusive and exhaustive work on the fascinating phenomenon variously known as mutation, saltation, dissociation, etc.—LEON H. LEONIAN, West Virginia University, Morgantown, West Virginia.

## ANNOUNCEMENT

The summer meeting of The American Phytopathological Society will be held jointly with the Pacific Division of the Society, in Denver, Colorado, June 23 to 26, inclusive. The first two days will be devoted to presentation of papers. On June 25, 10 A.M., those interested will visit potato experiment station at Greeley; lunch at Fort Collins; visit irrigation laboratory; Estes Park via Thompson Canyon, 2 P.M.; steak fry and campfire, Estes Park, 6:30 P.M.; lectures on irrigation and other problems of western agriculture. June 26, 9:30 A.M., tour over Trail Ridge road to Grand Lake; lunch at Grand Lake. Return to Denver via Berthoud Pass.

# HISTOLOGICAL STUDIES ON WILT OF CHINA ASTER<sup>1</sup>

ARNOLD J. ULLSTRUP

(Received for publication March 18, 1937)

## INTRODUCTION

The wilt disease of China aster, *Callistephus chinensis* Nees., caused by *Fusarium conglomerans* Wr. var. *callistephi* Beach, occurs throughout most of the temperate regions of the world wherever the host plant has become established. The earliest record of the disease in the United States is by Galloway (7), in 1896. He noted the similarity between this disease and other vascular wilts, and stated that the parasite probably enters the host near the soil line. Since that time considerable literature has accumulated on the subject. Beach (4), in 1918, made an extensive study of the symptoms of the disease and described and named the causal organism. In 1927, Jackson (8) reported investigations on aster wilt, which were essentially in agreement with the findings of Beach. A number of workers (1, 3, 4, and 8) have suggested that the most feasible means of controlling the disease is through the selection of resistant varieties. Jones and Riker (9) have directed particular attention to this control method. By constant selection over a period of years, they have developed varieties of asters that combine a high degree of resistance with desirable floral characteristics.

The experiments reported in the present paper were designed to determine the mode of entrance of the parasite into the host, the relation of the fungus to the tissue of the host after penetration, and whether resistant plants showed morphological differences such as might prevent the entrance or impede the progress of the parasite within the tissues.

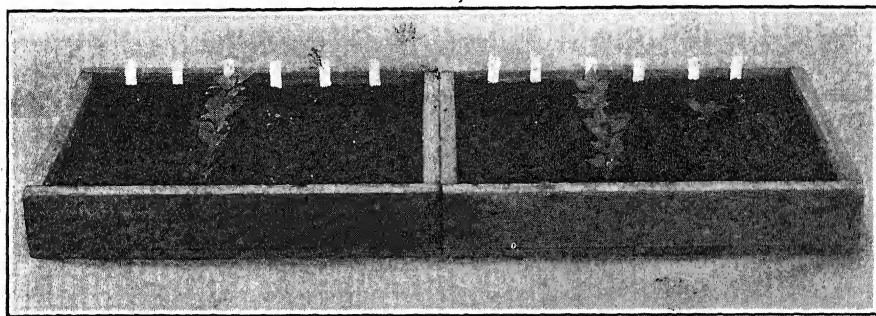
## MATERIALS AND METHODS

The varieties of China asters used were of both resistant and susceptible stocks. The resistant strains had been developed by Dr. L. O. Kunkel from a commercial variety through continued selection for several years. The susceptible varieties were procured from commercial sources. Tests of the relative susceptibility of the varieties were made in artificially contaminated soil maintained at a temperature of 20–22° C. in a greenhouse. The fungus used throughout the experiments was from a culture of *Fusarium conglomerans* var. *callistephi* originally isolated by Beach.<sup>2</sup> Soil, known to be free of the wilt organism, was contaminated by adding one part of a cornmeal-sand medium, on which the fungus was growing, to 6 parts of soil and allowing

<sup>1</sup> Published at the expense of The Rockefeller Institute for Medical Research, Princeton, N. J., out of the order determined by the date of receipt of the manuscript. This practice in no wise delays the publication of manuscripts printed at the expense of The American Phytopathological Society or other agency.

<sup>2</sup> This culture, No. 305, was obtained through the kindness of Dr. Regina S. Riker.

the mixture to stand for several days. Four-week-old seedlings of resistant and susceptible varieties, grown in quartz sand and fed with a nutrient solution, were then transplanted to the contaminated soil. Observations on the relative susceptibility of the varieties were made 4 and 8 weeks after transplanting. At the conclusion of the trials, about 80 to 90 per cent of the plants of resistant strains were growing vigorously; and of these a line designated as No. 14 was selected for subsequent experimentation. Most of the commercial varieties were found to be 100 per cent susceptible. The lowest susceptibility shown by any of these lines was about 98 per cent (Fig. 1).



(Photographs by J. A. Carlile)

FIG. 1. Results of an experiment on the relative resistance of aster varieties to wilt. The seedlings in the 3rd row from the left in each flat are of the resistant variety No. 14. The remaining 5 rows in each flat were planted with seedlings from commercial varieties. One plant of these remained healthy. The photograph was taken 6 weeks after 4-week-old seedlings were planted in the wilt soil.

The procedure finally adopted for the study of host penetration was as follows: Seeds were soaked for 15 to 18 hours in sterile distilled water and then immersed in a solution of mercuric chloride (1:1000) for 5 minutes. After rinsing several times in sterile water, the seeds were placed on hard potato-dextrose agar and allowed to germinate. Seeds that were free of contamination and at approximately the same stage of germination were dipped momentarily in a spore suspension of the fungus and planted in wilt soil held at 20–22° C. The conditions thus obtained appeared to be comparable to those found in a wilt-infested field and provided an environment suitable for a study of the differential of resistance between susceptible and resistant varieties. Other methods, which involved growing seedlings on soil-extract agar in the presence of the fungus, or in quartz sand, contaminated with the wilt organism proved unsatisfactory, since either seedling growth was abnormal or the differential between susceptible and resistant varieties was not clearly expressed.

Seedlings inoculated and grown according to the method described were carefully removed from the soil at 48-hour intervals and placed in fixing solution.



The fixative found most satisfactory consisted of 20 cc. of 1 per cent chromic acid, 75 cc. of 1 per cent acetic acid, and 5 cc. of commercial formaldehyde. After being in the fixative for 48 hours, the tissues were dehydrated, de-alcoholized with cedar oil, and imbedded in paraffin. Serial sections were cut  $8\mu$  to  $16\mu$  in thickness. The most satisfactory staining combination found was that reported by Moore (10) in which safranin and fast green were used.

Subsequent development of the parasite within the host tissues was studied on older seedlings grown in wilt-infested soil. Surface-sterilized seeds were planted in quartz sand and fed with a nutrient solution until 4 weeks of age. They were then carefully removed from the sand, dipped in a spore suspension of the fungus, and transplanted to the soil. Fixations of roots were made at 48-hour intervals for 3 weeks following inoculation.

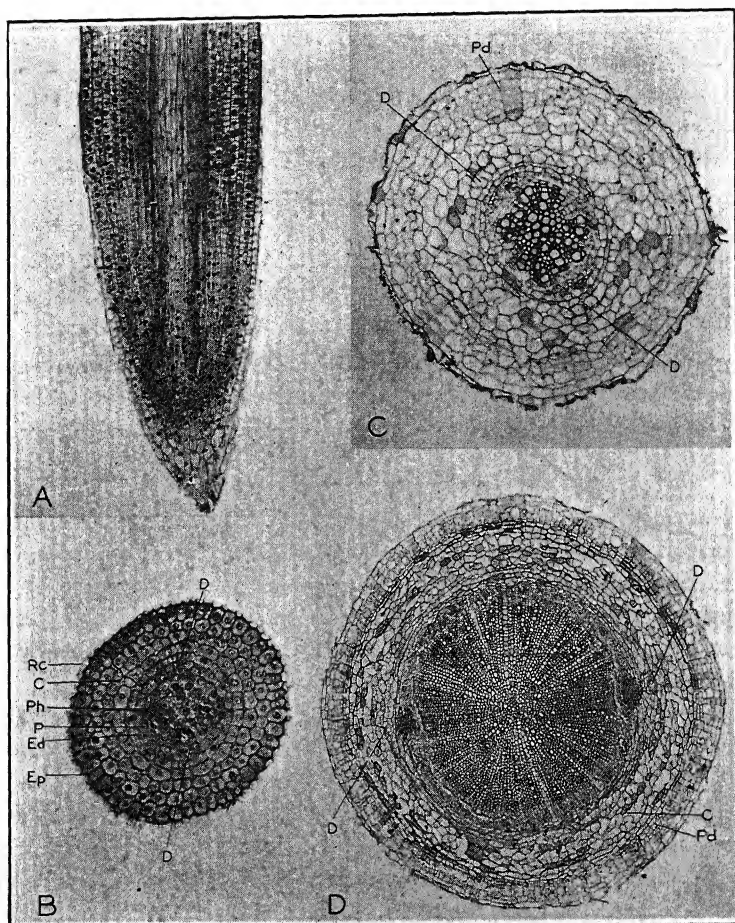
#### OBSERVATIONS

*Anatomy of the Healthy Root.* Since no report was found in the literature describing the anatomy of the normal root of the China aster, a study of the healthy root was made for the purpose of orientation in later studies on the pathological histology. Inasmuch as no morphological differences were observed between roots of resistant and susceptible varieties, it is unnecessary to describe them separately.

The embryonic region of the aster root (Fig. 2, A) is protected by the cap which is 5 to 8 cells thick at its apex and tapers off, as it ensheaths the anterior portion of this region, to a cylindrical structure 1 cell in thickness. The primary meristem, behind the root cap, extends posteriorly for a distance of about 1 mm. A cross section through the middle of the meristematic region (Fig. 2, B) shows a single layer of cells of the root cap, and immediately beneath these cells are the radially elongated cells of the epidermis.

The cortex is composed of 4 to 6 rows of cells. The prominent intercellular spaces in this tissue become increasingly larger with maturation of the root. A single row of cells makes up the endodermis. In the embryonic region Casparian strips are not distinguishable in the endodermis. Soon after secondary thickening is initiated, however, Casparian strips are discernible. When viewed in cross section, two regions of the endodermis diametrically opposite each other appear to be composed of a double row of cells. In the center of these double rows where the corners of 4 cells abut on one another, schizogenous ducts occur (Fig. 2, B, C). This condition is very similar to that found by Warden (13) in *Senecio vulgaris* L. When observed at a later stage, it can be seen that only the inner layer at these points shows Casparian strips, suggesting that the outer layer is not truly a part of the endodermis.

The pericycle lies inside the endodermis and is made up of a single row



(Photographs by J. A. Carlile)

FIG. 2. Sections of healthy aster roots at various stages of development. A. Longitudinal section of root tip showing the cap and embryonic region.  $\times 174$ . B. Cross section through embryonic region showing early differentiation of tissues: Rc, root cap; Ep, epidermis; C, cortex; Ed, endodermis; D, ducts; P, pericycle; Ph, sieve tubes.  $\times 215$ . C. Cross section of root about the time of secondary thickening, showing development of periderm, Pd. Ducts shown at D.  $\times 323$ . D. Old root showing well-developed ducts, D, periderm, Pd, and cortex, C.

of isodiametric cells containing dense cytoplasm. Secondary roots arise in this tissue between the points of the protoxylem and protophloem.

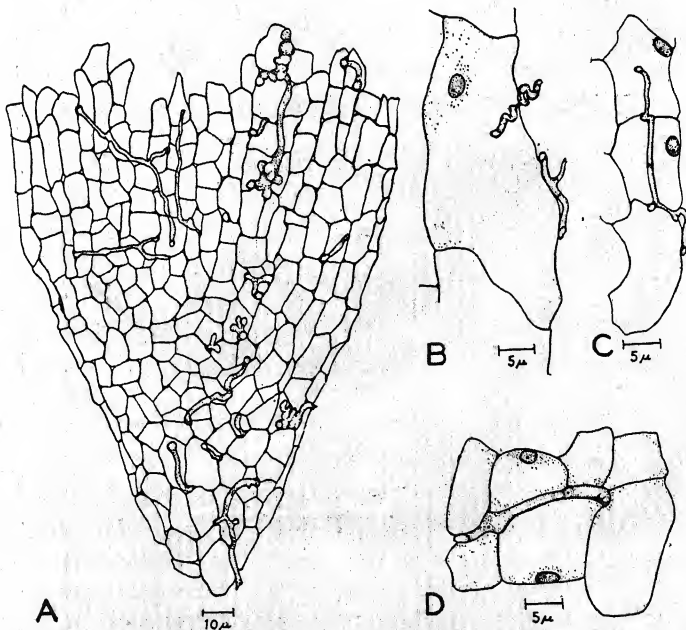
The sieve tubes are the first of the vascular tissues to differentiate. They are found just within the pericycle and directly opposite the endodermal ducts (Fig. 2, B). Shortly after the appearance of sieve tubes, the heavy-walled cells of the protoxylem differentiate. The xylem is arranged in a diarch condition; in a few instances, however, a triarch condition was found. In the old portion of the root, at the time secondary thickening has begun,



a periderm layer is formed in the cortex, beneath the epidermis (Fig. 2, C). As the periderm develops, the epidermis is sloughed off, leaving the phellem 4 to 6 cells in thickness, as a protective layer.

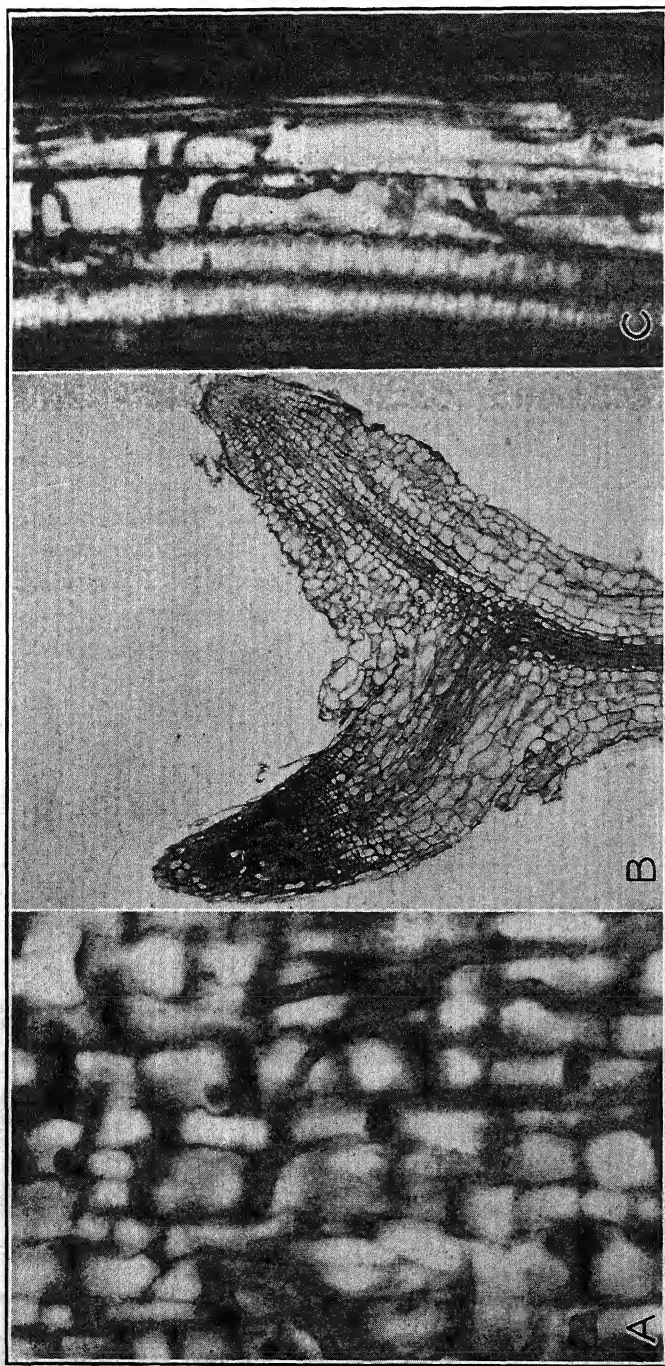
The mature root is composed largely of woody secondary xylem surrounded by a complete sheath of cambium. Exterior to the cambium is the phloem interspersed with heavy-walled fibers. Opposite the points where the protophloem first differentiated, and where in the mature root the phloem fibers are most abundant, are well-defined ducts in the endodermis. The relatively thin cortex is retained throughout the life of the plant and is protected by the periderm (Fig. 2, D).

*Penetration.* The first stages of penetration by the parasite were observed in roots fixed 4 to 8 days after inoculation. The point where penetration was most frequently found to take place was the root cap. On the exterior of the cap, dead sloughed cells and knotted masses of hyphae were often seen. It is possible that these dead cells furnished a rich substrate for saprophytic development of the fungus and thus provided large amounts of inoculum at a particularly vulnerable point. Here the hyphae penetrated between the cells and eventually entered the embryonic region (Fig. 3, A). Not infrequently hyphae were observed penetrating between the epidermal cells in the region of elongation (Fig. 3, C). In a few instances direct



(Photographs by J. A. Carlile)

FIG. 3. A. Hyphae in the cap and embryonic region of a diseased root tip. B. Direct penetration of an epidermal cell. C. Intercellular penetration. D. Intercellular development of hyphae after penetration.



(Photographs by J. A. Carille)

FIG. 4. A. Development of hyphae in the embryonic region of a diseased root.  $\times 1400$ . B. Branching at an abnormally short distance from root tip. The primary root tip at the right is diseased, the branch at the left is healthy.  $\times 84$ . C. Advanced stage of infection of a young seedling showing hyphae in xylem vessels.  $\times 728$ .

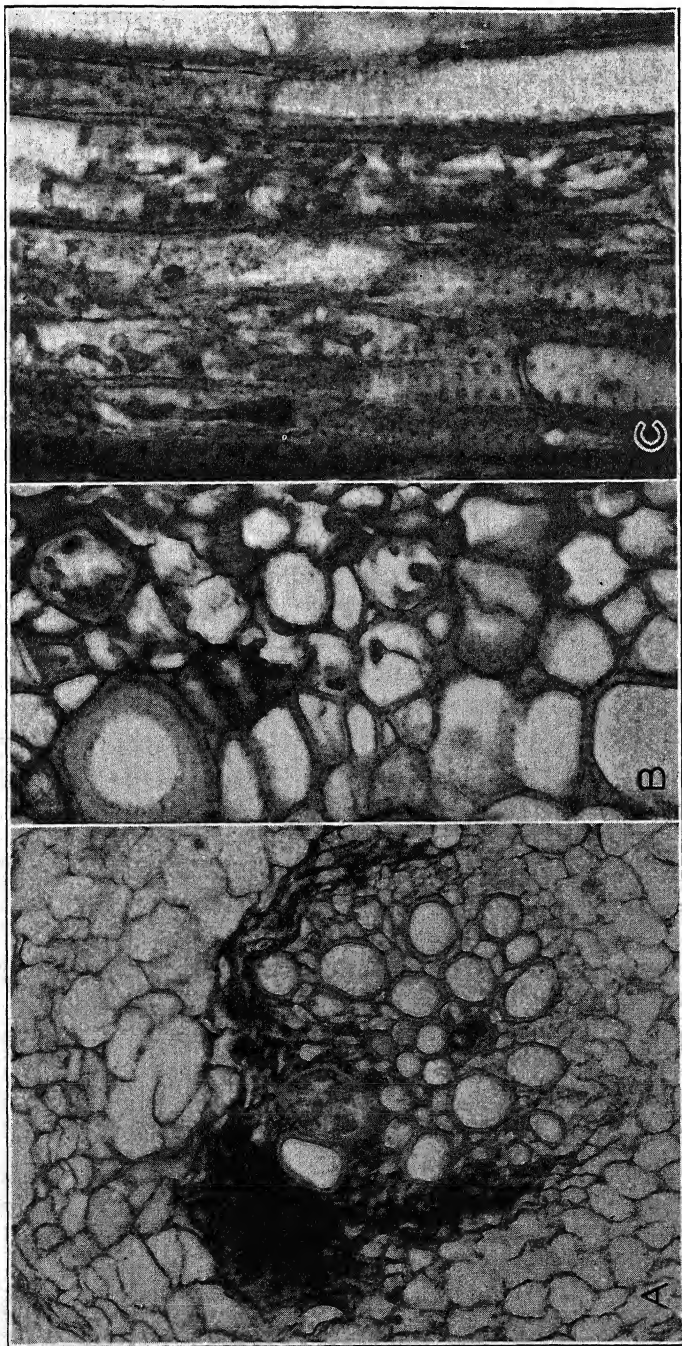


FIG. 5. A. Heavy infection of young root showing fungus in the xylem, phloem, and pericycle. Note infection of primordium of secondary root at upper left.  $\times 533$ . B. Early development of hyphae in xylem of older root.  $\times 600$ . C. Advanced infection in xylem of older root.  $\times 600$ .

(Photographs by J. A. Caritile)

penetration of the wall of an epidermal cell was observed (Fig. 3, B), but such cases occurred only in roots where the mycelium was already present within the tissues. This mode of penetration may have been due to lowered resistance brought about by an earlier penetration.

In none of the sections observed was initial penetration found to take place through root hairs. Hyphae were occasionally found in root hairs where the fungus had invaded the tissues extensively. Such hair cells were probably dead or in a state of low vigor when the fungus entered them.

Once the root tip was penetrated, host cell nuclei ceased to divide and became slightly plasmolyzed and nearly hyaline. After invasion of the meristematic region, secondary roots frequently arose at an abnormally short distance behind the tip (Fig. 4, B). This condition was apparently a response to penetration, since in normal roots grown in wilt-free soil, branching occurred at a much greater distance from the tip.

In roots of resistant seedlings very little penetration was observed. The majority of these seedlings were entirely free of the fungus, but where infection was found the manner in which it took place and the points at which the hyphae entered were the same as in seedlings of susceptible varieties.

After penetrating the young root tip of a susceptible seedling, the fungus progressed both inter- and intracellularly throughout the tissues (Fig. 3, A, D; Fig. 4, A). Occasionally the entire embryonic region and part of the region of elongation were so overrun by the parasite that little of the host tissues could be recognized. This complete disorganization of the host tissue in the early stages of the disease was generally limited to the region of elongation. Posterior to this region the mycelium was confined largely to the xylem elements (Fig. 4, C).

*Development of the Parasite in Old Portions of the Root.* Healthy 4-week-old seedlings of resistant and susceptible varieties were transplanted to wilt-infested soil and fixations of the roots made at 48-hour intervals.

Sections of root tips from susceptible plants showed penetration to take place in a similar manner and at the same points as in the primary root of younger seedlings. Likewise, the progress of the fungus through the immature tissues was no different from that in the seedlings grown in wilt soil from the time of germination.

In the old maturing tissues the fungus was found in the vessels of the xylem, and it was through these channels that the organism made its greatest advance from the point of infection (Fig. 5, B). Two or 3 weeks after inoculation, the mycelium was found in abundance not only in the xylem but also in the cambium and phloem (Fig. 5, A). Most of the xylem vessels contained some hyphal strands (Fig. 5, C), but few were completely plugged. Gum-like substances were often observed in the xylem vessels of roots that were heavily infected.

Diseased lateral roots contributed to the severity of infection of primary roots. Lateral roots were in turn often infected internally by mycelium that had grown from the stele of the main root. The infection of the primordium of a secondary root by such means is shown in figure 5, A.

Sections of old roots from resistant plants showed comparatively few cases of infection. In these few cases the behavior of the fungus and the reaction of the host to invasion was in no way distinguishable from that in susceptible plants.

In order to study infection of resistant plants in more detail, and to eliminate the few plants in the resistant variety that normally succumb to wilt, the following experiment was set up: Four-week-old seedlings of resistant and susceptible varieties were transplanted to wilt soil. After 3 to 4 weeks, all of the plants of the susceptible variety were either dead or severely wilted. About 10 to 15 per cent of the resistant plants had wilted. Three months later, the remaining resistant plants were removed from the soil, their roots carefully washed free from debris and examined for signs of invasion by the wilt fungus. Aside from a few discolored lesions, the root systems appeared normal and comparable to those grown in wilt-free soil. The lesions were very much restricted in extent and were localized at points where short, lateral roots, 5 to 10 mm. in length, arose. These symptoms suggested that infection may have taken place through the tips of the lateral roots or through the rupture made by their emergence. Prior to fixation of roots bearing lesions, small bits of tissue from discolored areas were surface-sterilized and plated out on acidified potato-dextrose agar to determine whether or not the lesions were caused by the wilt organism. In practically all cases the platings gave pure cultures of the fungus under consideration. Sections through such lesions and the immediately adjoining, healthy-appearing tissue, and also sections through portions of the secondary and main roots at varying distances from the infection were prepared.

Penetration was found to take place through the cap of these short lateral roots in the same manner as in susceptible roots. The fungus in some cases had advanced a few millimeters in the root from which the lateral arose, but here little further development was noted. Although these discolored areas of infection were restricted, nothing of the nature of a morphological barrier that might act in delimiting the progress of the parasite was found. The hyphae were in no way distorted or abnormal in these regions, nor was there any apparent reaction on the part of the host cells that would suggest an antagonistic response to invasion. Examination of sections cut from apparently healthy tissues at varying distances from the lesions showed the fungus to be absent and the host cells in a normal condition.



## DISCUSSION

In the present investigation it has been shown that penetration of roots of susceptible varieties of China asters by the fungus *Fusarium conglomerans* var. *callistephi* takes place between the cells of the root cap and between the epidermal cells in the region of elongation. In a few instances direct penetration of the outer wall of the epidermal cells was observed. Only in advanced stages of infection were hyphae occasionally seen in root hairs, and it is suggested that such penetration may have occurred after death or weakening of the hair cells due to an earlier penetration of the root through the observed avenues of entrance of the parasite.

Few of the young seedlings of the resistant aster variety used in this study showed signs of fungal invasion. The number that was found to be parasitized was approximately the same as the number that ordinarily succumbs when this variety is planted in wilt soil. Among older plants of the resistant variety, in which resistance was indicated by ability to grow normally in infested soil for over 3 months, a few restricted lesions caused by the wilt fungus were found. Although penetration and early development of the parasite within the tissues of these resistant plants was the same as found in susceptible varieties, the extensive progress of the fungus observed in susceptible plants was not found. The means by which the hyphae were prevented from completely overrunning the host could not be determined histologically. Neither structural barriers which might prevent advance of the fungus, nor peculiarities in the staining reaction of the host tissue that would indicate an antagonism between the cell contents and the parasite were observed.

Smith and Walker (11), in a histological study of cabbage yellows, found no morphological differences to exist between homozygous resistant and homozygous susceptible plants. In addition, penetration of the resistant host did not stimulate the formation of tissues to obstruct development of the fungus. Anderson and Walker (2) studied the histology of cabbage yellows in lines of plants in which resistance was governed by multiple factors. They found no definite morphological basis for resistance, but did observe certain host responses to penetration. Cell walls became suberized slightly in advance of the fungus, and granulation of the cell contents often occurred upon penetration. Although such responses occurred with greater frequency in the resistant host, the authors concluded that reactions of this kind could not be regarded as the basis for the marked difference in resistance between the lines of the host plant employed. Except for the host responses noted by Anderson and Walker, the penetration and subsequent development of the cabbage yellows organism in its host is in many respects similar to that found in the present investigation on the aster wilt fungus.

Tisdale (12), working with flax wilt, found that a cork barrier was laid

down in response to penetration, but he suggested undetermined physiological factors as probably playing an important rôle in resistance to this disease.

Dharmarajulu (6), in a histological study of resistance in cotton to *Fusarium vasinfectum* Atk., concluded that the combined action of suberized cell walls and antagonistic effects of the host protoplasm on the fungus was responsible for resistance.

Conant (5) has shown very definitely that resistance in tobacco to the cortical-rotting fungus, *Thielavia basicola* Zopf, is based upon the ability of the host to form a cork layer that inhibits further advance of the mycelium.

In the study of wilt diseases caused by vascular-invading *Fusaria*, comparative histological investigations have failed to show any morphological differences between the healthy plants of resistant and susceptible lines. Furthermore, resistant plants are not stimulated upon penetration to form mechanical barriers that are alone able to prevent the development of the disease-producing organism. Resistance to these wilt diseases is expressed at the points of invasion, in undifferentiated or immature tissues, where mechanical barriers obstructing the progress of the parasite are not to be found.

In the present studies, the resistance shown by certain varieties of asters does not appear to be connected with any morphological structures. It is probably associated with the physiological nature of the host protoplast.

#### SUMMARY

The normal and pathological anatomy of roots of varieties of China asters resistant and susceptible to wilt was studied.

Anatomically, the roots of healthy resistant plants were indistinguishable from those of healthy susceptible varieties.

Penetration of susceptible plants takes place largely between the cells of the root cap and between the epidermal cells in the region of elongation. Occasionally, direct penetration of the outer wall of an epidermal cell was observed. Initial penetration through root hairs was not observed.

In the resistant strain very little penetration was found. Among those roots of this line that were invaded, the mode of penetration and points at which it took place were the same as in susceptible varieties.

Progress of the fungus following penetration was studied in old susceptible seedlings planted in wilt soil. Considerable rotting of the meristem and part of the region of elongation was observed. The hyphae, however, were soon confined to the xylem, and it was in this tissue that the fungus made its greatest progress. In advanced stages of the disease the mycelium extended into other stelar tissues and in so doing infected secondary roots. Until complete wilting ensued, very little of the cortex became involved.

Plants of the resistant variety grown in wilt soil for over 3 months showed

a few restricted lesions on their roots. No morphological barrier was observed that might act in delimiting the advance of hyphae in such lesions.

From a histological standpoint, aster wilt resembles other wilts caused by vascular *Fusaria*. Resistance appears to depend upon the physiological nature of the host protoplast and not upon mechanical structures existing either before penetration or developed after invasion.

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PRINCETON, NEW JERSEY

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# LONGEVITY OF GIBBERELLA SAUBINETII AND OTHER FUNGI IN BARLEY KERNELS AND ITS RELATION TO THE EMETIC EFFECT<sup>1</sup>

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Occasional crops of barley in local or general areas of the Midwestern States have been severely damaged by scab, a disease caused by the fungus, *Gibberella saubinetii* (Mont.) Sacc. Several investigators (5, 6, 7, 9) have shown that barley as a hog feed may be more or less impaired by the scab fungus, which produces in the grain an emetic principle tolerated by hogs in only small amounts. The chemical nature of the emetic has not been determined. The amount of the emetic principle usually is related to the percentage of infected kernels and degree of infection. The percentage of kernels plating the scab fungus has been used to indicate whether or not barley could be safely fed. However, occasional lots of barley more than a year old have been encountered in which the percentage of kernels plating *G. saubinetii* has been too low to account for the amount of the emetic principle as determined in feeding experiments. This condition apparently has resulted from a loss in viability of the fungus in a part of the infected kernels. The use of the plating method to determine the percentage of infected kernels and to discriminate between fungi causing discolored and blighted kernels is reliable, for the most part, insofar as the fungi retain their viability. The investigation here reported, therefore, was undertaken to determine the rate of decline in viability and longevity of *G. saubinetii* and other fungi in barley kernels and the possible relation of these factors to the limits of reliability of the plating test. A drenching test with pigs was made to determine whether or not the emetic principle in the kernels remained active after *G. saubinetii* had become nonviable.

Barre (1), in his work with cotton anthracnose, a seed-borne disease caused by *Glomerella gossypii*, found that in badly diseased seed the fungus remained viable for 12 to 15 months. However, he found that in slightly diseased seed the fungus was viable at the end of 2 years and, in a few cases, until that of the third year. In his work with bean anthracnose, Barrus (2) obtained cultures of *Colletotrichum lindemuthianum* from 2-year-old bean seed. Christensen (3) isolated *Helminthosporium sativum* in the spring of

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1921 from barley kernels grown in 1914, showing that the fungus remained viable for approximately 7 years in barley kernels. Leukel *et al.* (4) obtained 7 per cent of stripe-diseased plants from 5-year-old barley seed, which result showed that *H. gramineum* remained viable in barley kernels for at least 5 years.

#### MATERIAL AND METHODS

In this study 2 experiments were conducted with grain from Oderbrucker (Wisconsin Pedigree 6) barley plants inoculated with *Gibberella saubinetii* in 1930 in the first test and in 1931 in the second. In both years the method of inoculation was similar. The plants were covered with muslin cages from flowering to maturity and were inoculated every second day with a conidial suspension of *G. saubinetii* until it appeared that the plants were too near maturity for further infections to occur. The plants also were exposed to ascospore inoculum from corn stalks lying between the rows of barley. The tops of the cages were sprinkled with water several times during the day in order to maintain a high humidity within the cages. As a result, a large percentage of the kernels was infected with *G. saubinetii*.

In the first test the barley was harvested in early August, 1930, and the grain was divided into 2 lots for plating. One lot was obtained from the row that seemed most severely scabbed. The other was a composite from a number of rows. These 2 lots of grain were stored in paper bags and, throughout the experiment, were kept dry at room temperature in the laboratory. In the second test the grain was harvested in early August, 1931. The latter sample was a composite from a number of rows from scattered locations through the cages. After the first plating, the sample was divided into 2 lots. Both lots were stored in paper bags; one lot in the laboratory at room temperature, the other at the University-farm seed house. Lots stored in the laboratory were subjected to narrow fluctuations of temperature, while the lot stored in the unheated seed house was subjected to a wide range of temperature. Storage in the latter probably was not entirely comparable to that in a farm bin.

At various intervals after harvest, in both experiments, platings were made of 100-kernel samples taken at random after the seed lot had been mixed. The kernels were treated for approximately 20 minutes with a 10 per cent solution of B-K<sup>3</sup> to eliminate surface contaminations so that internal infections, probably in the mycelial form, would be indicated in platings.

The method of surface sterilization employed in this work was tested for its effectiveness on spores of *Gibberella saubinetii*. Scabbed barley kernels were used for this test after storage in the laboratory for 9 months after harvest. At that time germination percentages of mature conidia and ascospores from nonsterilized kernels occurred in very low percentages. Kernels

<sup>3</sup> B-K, a commercial product, is a 3.5 per cent solution of sodium hypochlorite.

were surface-sterilized for 20 minutes in a 10 per cent solution of B-K. This treatment inhibited germination of spores of *G. saubinetii*, including surface conidia and ascospores in perithecia; also, conidia located between the hull and the caryopsis. Mycelium grew out readily from lemmas after the surface sterilization. Mycelium developed readily also from kernels that were surface-sterilized after removal of the hulls.

The above results showed that surface treatment with 10 per cent B-K for 20 minutes was sufficient to kill conidia and ascospores on the surface, as well as conidia found between the pericarp and the hull. Mycelium imbedded within the tissues of the lemma and palea and within the seed developed readily after surface sterilization.

Platings were made on potato-dextrose agar, acidified with lactic acid in order to discourage bacterial growth. The plates were incubated at room temperature for 4 to 10 days or longer, depending upon the time necessary for the fungi to fruit. Bacterial colonies very seldom succeeded in developing from kernels. Where both a fungus and a bacterial colony developed from the same kernel, only the fungus was recorded.

PLATING RESULTS FROM BARLEY INOCULATED WITH *GIBBERELLA*  
*SAUBINETII* IN 1930

Frequently 2 and sometimes 3 different fungi were plated from a single kernel. Each was recorded under its appropriate genus. Therefore, the total number of kernel infections and the number of sterile kernels exceeded 100 in the early platings of each barley lot. The most frequent associations of fungi in a kernel were *Gibberella saubinetii* with either *Alternaria* or *Helminthosporium*.

Plating results of barley kernels from the lot of composite rows harvested in 1930 are given in table 1.<sup>4</sup> Since the data from the most severely scabbed row did not differ significantly from those in table 1, they are not given in tabular form. The curves in figure 1, A, show that, at the different dates of plating, the percentages of kernels with viable *Gibberella saubinetii* were approximately the same in the 2 lots of barley. The 2 curves are given in order to show the close parallelism and to show the maximum longevity of the fungus recorded in these experiments. The initial percentage of infection was very high in both lots. Since the lot from the most severely scabbed row plated 98 per cent of *G. saubinetii* on February 12, 1931, the fungus could have lost very little, if any, of its viability up to that date. The same lot plated only 1 per cent on October 20, 1932, 27 months after harvest. *G. saubinetii* was found nonviable in this lot on January 19, 1933, 30 months after harvest; throughout the remaining platings, the fungus failed to ap-

<sup>4</sup> The author acknowledges the aid in identification of fungi given by Miss Helen Johann, of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

TABLE 1.—Results of platings at various intervals of random 100 kernel samples of *Oderbrucker barley* artificially inoculated with *Gibberella saubinetii* in 1930 and stored in the laboratory from 1930 to 1935 at Madison, Wis.

Date plated	Number of months after harvest	Number of kernels plating						Number of sterile kernels
		<i>Gibberella saubinetii</i>	<i>Alternaria</i>	<i>Helminthosporium</i>	<i>Penicillium</i>	<i>Basipyrum</i>	<i>Fusaria</i> <sup>a</sup>	Miscellaneous <sup>b</sup>
4/10/31	8	91	5	2	1	0	0	3
5/7/31	9	92	14	5	0	0	2	0
6/18/31	11	87	13	1	0	0	1	1
8/26/31	13	49	24	1	1	1	6	22
12/7/31	16	18	16	1	0	2	1	61
2/3/32	18	6	10	3	0	1	0	80
3/21/32	20	8	14	5	1	0	1	68
5/9/32	21	4	11	2	2	0	1	78
10/20/32	27	0	5	4	1	1	0	85
1/19/33	30	0	3	2	0	0	0	95
3/21/33	32	0	4	0	0	0	0	94
2/13/34	42	0	5	2	0	0	0	91
11/11/34	51	0	1	2	0	0	0	90
5/6/35	57	0	1	0	0	0	1	96
10/19/35	63	0	0	0	0	0	0	96

<sup>a</sup> *Fusaria* other than the conidial stage of *G. saubinetii*.

<sup>b</sup> Miscellaneous and unidentified fungi and bacteria.

<sup>c</sup> Bacteria.

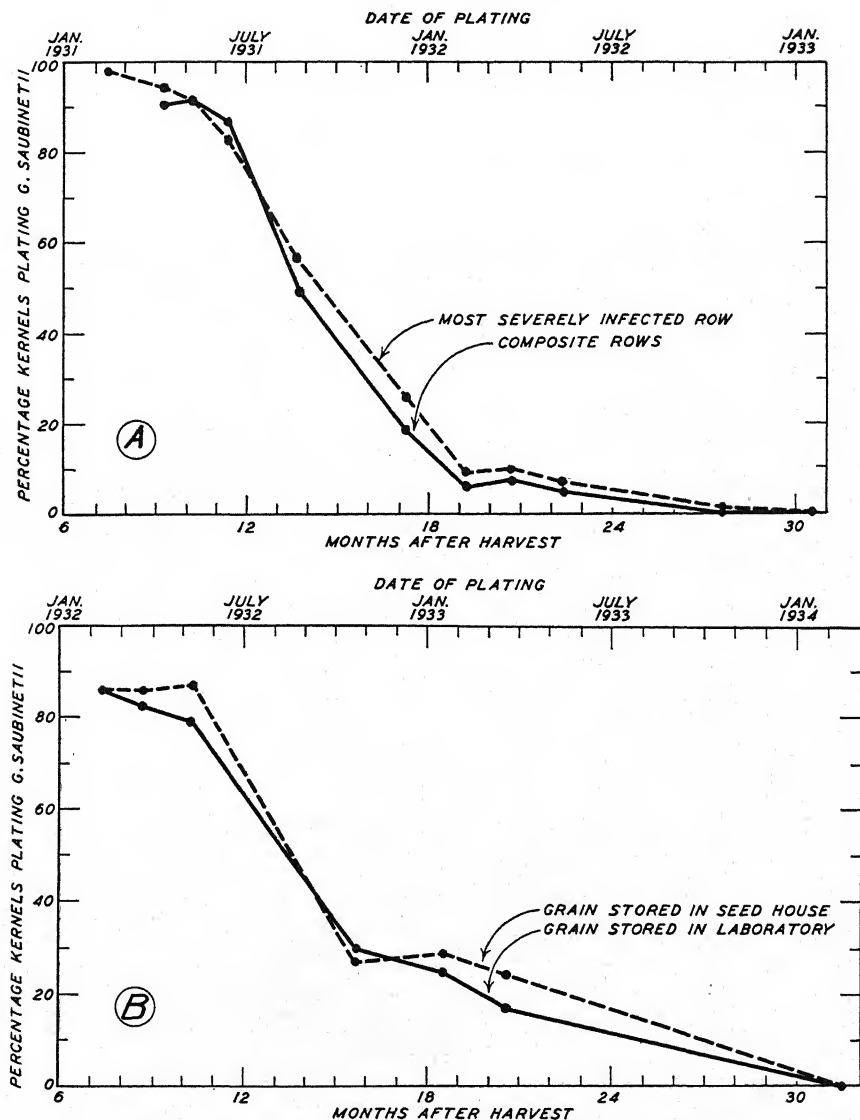


FIG. 1. Rate of decline in the percentage of kernels plating *Gibberella saubinetii* from Oderbrucker (Wisconsin Pedigree 6) barley artificially inoculated with *G. saubinetii*. A. Grown 1930 in scab cage. Two samples stored in the laboratory from 1930 to 1935. B. Grown 1931 in scab cage. One lot stored in the laboratory from 1931 to 1935; the other lot stored in the laboratory until February, 1932, and then in the seed house from 1932 to 1935.

pear. The lot of barley from the composite rows plated 91 per cent of *G. saubinetii* on April 10, 1931, and 4 per cent on May 9, 1932, 21 months after harvest. The fungus was nonviable in kernels on October 20, 1932,

27 months after harvest, and did not appear in any of the subsequent platings of this lot of barley. As shown in the curves of figure 1, A, the rate of loss in viability of *G. saubinetii* was similar in the 2 lots. The drop in viability was noticeable by June, 1931, approximately 11 months after harvest. Of the kernels plating *G. saubinetii* in samples from the 2 lots of barley on April 10, 1931, the fungus remained viable in approximately 57 per cent of the kernels at 13 months, 24 per cent at 16 months, 8 per cent at 18 months, 0.54 per cent at 27 months, and had completely lost its viability at 30 months after harvest. The most rapid drop in viability occurred between the 11th and 18th months after harvest, during which period *G. saubinetii* lost its viability in approximately 84 per cent of the kernels that plated the fungus on April 10, 1931.

The percentage of kernels plating *Alternaria* ranked second to that of *Gibberella saubinetii* during the early period of the experiment. Table 1 shows that the percentage of kernels plating *Alternaria* increased for 13 months after harvest during which period *G. saubinetii* decreased. The percentage of kernels plating *Alternaria* increased as *G. saubinetii* decreased somewhat in vigor and viability. After 13 months, the viability of *Alternaria* gradually decreased until, at 57 months after harvest, the fungus remained viable in only 1 per cent of the kernels; and at 63 months, it did not grow from the plated kernels.

The *Helminthosporium* species that produce conidia in culture were plated from a small percentage of the kernels. These fungi retained their viability for 51 months after harvest and failed to appear in 2 subsequent platings. *Penicillium* and *Basisporium* were plated in small percentages, neither of which appeared after 27 months. No attempt was made to classify the several species of *Fusarium* plated. Probably the section *Sporotrichiella* was represented most often. One kernel plated an unidentified species of *Fusarium* at 57 months after harvest. Occasionally, *Aspergillus* and *Cephalothecium* were plated and recorded with the miscellaneous group. In nearly all platings there were a few fungi that failed to fruit and also were recorded with the miscellaneous group. One of these produced a grayish or brownish mycelium, suspected of being *Helminthosporium gramineum* Rabh. On October 19, 1935, 4 kernels plated fungi that failed to fruit and were recorded with the miscellaneous group. Barley seed was inoculated with cultures from the 4 kernels and 2 of the cultures produced stripe-diseased plants. The noninoculated control was disease-free.

As the fungi in the infected kernels lost their viability, there was a corresponding increase in the number of sterile kernels. The percentage of sterile kernels increased from 3 per cent at 8 months after harvest to 95 per cent at 30 months; thereafter, the sterile kernels fluctuated from 91 per cent to 96 per cent.

PLATING RESULTS FROM BARLEY INOCULATED WITH *GIBBERELLA SAUBINETII*  
IN 1931

Grain from the composite of Oderbrucker rows grown in the scab cages in 1931 was divided into 2 lots and stored at 2 places in order to determine whether or not storage conditions affected the longevity of fungi in the grain. The lots of grain were kept dry through the period of storage. Plating results of the lot stored in the laboratory are given in table 2, and the results from the lot stored in the University-farm seed house, in table 3. The first plating in each of these tables is the same plating made before the sample was divided.

The first plating showed that 86 per cent of the kernels contained viable *Gibberella saubinetii*. The difference in rate of loss of viability for the 2 lots of barley is shown by the curves in figure 1, B. On May 9, 1932, the viability of this fungus had not decreased in the barley stored in the seed house, while at the same time the fungus was plated from 79 per cent of the kernels in the laboratory lot, a decrease of 8 per cent in viability. Between May 9 and October 20, 1932, or between the 9th and 15th month after harvest, viability dropped very rapidly. On October 20, 1932, *G. saubinetii* was plated from 30 per cent of the kernels in the laboratory lot and 28 per cent in the seed house lot, a decrease in viability from the first plating of 65 and 67 per cent, respectively. On March 21, 1933, the fungus was plated from 17 per cent of the kernels stored in the laboratory and from 24 per cent of those stored in the seed house, a decrease in viability from the first plating of 80 and 72 per cent, respectively. At the next plating on February 13, 1934, or approximately 30 months after harvest, *G. saubinetii* was non-viable in both barley lots and failed to appear in later platings. The curves in figure 1, B, show that during the winter months very little loss in viability occurred in the lot stored at the seed house, while there was an unmistakable decline in the lot stored at the laboratory.

*Alternaria*, found in 26 per cent of the kernels in the first plating, fluctuated considerably in platings from subsequent samples of both barley lots. Higher percentages of this fungus usually were plated from the laboratory lot than from the lot stored at the seed house. In the former, 1 per cent of the kernels plated *Alternaria* 51 months after harvest. The fungus was nonviable in kernels plated later than 33 months after harvest from barley stored in the seed house.

The *Helminthosporium* species that produce conidia in culture were plated in consistently higher percentages and remained viable longer in the laboratory lot than in the lot stored at the seed house. The fungi in this genus retained their viability for 39 months in the laboratory lot and 33 months in the lot stored in the seed house.

*Penicillium*, which was plated from only a few kernels, was viable as late



TABLE 2.—Results of platings at various intervals of random 100 kernel samples of Oederbrucker barley artificially inoculated with *Gibberella saubinetii* in 1931 and stored in the laboratory from 1931 to 1935 at Madison, Wis.

Date plated	Number of months after harvest	Number of kernels plating							Number of sterile kernels
		<i>Gibberella saubinetii</i>	<i>Atter-naria</i>	<i>Helmintho-sporium</i>	<i>Penicil-lium</i>	<i>Basispo-rium</i>	<i>Fusaria</i> <sup>a</sup>	Miscel-laneous <sup>b</sup>	
2/10/32	6	86	26	22	1	0	3	4	0
3/21/32	8	82	12	6	1	0	1	1	6
5/ 9/32	9	79	9	4	1	3	0	2	6
10/20/32	15	30	10	22	1	3	4	5	31
1/19/33	18	25	17	16	0	1	3	0	40
3/21/33	20	17	5	9	0	0	4	1	64
2/13/34	30	0	5	14	2	0	2	0	78
5/14/34	33	0	1	16	0	0	3	1	80
11/11/34	39	0	1	2	0	0	1	1+8 <sup>c</sup>	87
5/ 6/35	45	0	2	0	0	0	0	3	95
10/19/35	51	0	1	0	0	0	1	1	97

<sup>a</sup> Fusaria other than the conidial stage of *G. saubinetii*.

<sup>b</sup> Miscellaneous and unidentified fungi and bacteria.

<sup>c</sup> Bacteria.



TABLE 3.—Results of platings at various intervals of random 100 kernel samples of *Oderbrucker barley* artificially inoculated with *Gibberella saubinetii* in 1931 and stored in the seed house of the University farm from 1932–1935 at Madison, Wis.

Date plated	Number of months after harvest	Number of kernels plating						Number of sterile kernels	
		<i>Gibberella saubinetii</i>	<i>Alter-naria</i>	<i>Helmintho-sporium</i>	<i>Penicil-lum</i>	<i>Basispo-rium</i>	<i>Fusaria</i> <sup>a</sup>		Miscel-laneous <sup>b</sup>
2/10/32c	6	86	26	22	1	0	3	4	0
3/21/32	8	86	6	3	0	3	0	3	2
5/ 9/32	9	87	8	2	0	0	0	2	2
10/20/32	15	28	10	21	1	2	6	7 + 4d	31
1/19/33	18	29	5	14	0	2	2	4	46
3/21/33	20	24	8	7	0	2	0	0	60
2/13/34	30	0	6	9	0	0	1	1	83
5/14/34	33	0	5	2	0	0	1	1 + 3d	88
11/11/34	39	0	0	0	0	0	3	1 + 12a	85
5/ 6/35	45	0	0	0	0	0	0	0	100
10/19/35	51	0	0	0	0	0	0	0	100

<sup>a</sup> Fusaria other than the conidial stage of *G. saubinetii*.

<sup>b</sup> Miscellaneous and unidentified fungi and bacteria.

<sup>c</sup> The barley used for plating studies given in tables 2 and 3 was stored in the laboratory until February, 1932, when a plating was made and the sample divided into 2 lots and stored as indicated in the headings. The data of the first plating are included in both tables in order to facilitate comparisons.

<sup>d</sup> Bacteria.

as 30 months and 15 months after harvest in lots stored in the laboratory and seed house, respectively. *Basisporium* remained viable for 18 months in barley stored in the laboratory and for 20 months in that stored in the seed house. One kernel plated an unidentified *Fusarium* in the laboratory lot at 51 months after harvest and, in the lot stored in the seed house, *Fusaria* were plated at 39 months after harvest. In the laboratory lot, 97 per cent of the kernels were free from viable bacteria and fungi at 51 months, whereas 100 per cent of the kernels from the seed-house lot appeared sterile in the plating at 45 months after harvest.

#### PLATINGS FROM FIELD LOTS OF BARLEY

A sample of fairly plump blight-damaged barley, grown in 1929, was obtained from Missouri Valley, Iowa, and the plating results are given in table 4. Approximately 44 per cent of the kernels plated *Gibberella saubinetii* in November, 1929, when the seed was stored in the laboratory. In March, 1931, 20 months after harvest, this fungus was nonviable. During the same period, *Alternaria* had not lost in viability. In November, 1935, 75 months after harvest, 1 per cent of the kernels plated *Alternaria*. None of the species of *Helminthosporium* that produce conidia in platings was found at 20 months after harvest.

A sterile fungus which produced reddish brown mycelium on potato-dextrose agar was obtained from a few kernels of this barley. This type of fungus was plated from one kernel in the first plating, from 8 kernels

TABLE 4.—Plating results of random kernels taken from a commercial sample of barley grown at Missouri Valley, Iowa, in 1929. Stored in the laboratory

Date plated	Number months after harvest	Number kernels plated	Number of kernels plating						Number of sterile kernels
			<i>Gibberella saubinetii</i>	<i>Alternaria</i>	<i>Helminthosporium</i>	<i>Aspergillus</i>	<i>Fusaria</i> <sup>a</sup>	Miscellaneous <sup>b</sup>	
11/27/29	4	94	41	72	5	0	2	6	0
3/18/31	20	100	0	76	0	2	0	20	10
11/ 7/35	75	100	0	1	0	0	0	19	80

<sup>a</sup> *Fusaria* other than the conidial stage of *G. saubinetii*.

<sup>b</sup> Miscellaneous and unidentified fungi.

in the second plating, and from 12 kernels in the last plating, all of which were placed with the miscellaneous fungi. Out of the 12 kernels plating this type of fungus on November 7, 1935, 3 representative cultures were transferred for further study. These cultures appeared similar to the stripe organism and were tested for pathogenicity by inoculating barley seed. Each of these cultures was pathogenic, producing 31, 39, and 53 per cent

of typically stripe-diseased plants which showed that *Helminthosporium gramineum* remained viable in barley kernels for 75 months after harvest.

In a lot of Oderbrucker (Wis. Ped. 6) barley grown at Janesville, Wis., in 1925, 16 per cent of the plants developed stripe in field experiments conducted in 1928 at Madison. The seed lot was stored in the University-farm seed house between 1925 and 1929, and, after that, in the laboratory. A sample of 200 kernels taken at random from the 1925 seed was plated on November 5, 1935, as in previous experiments. The following percentages were obtained: 81.5 per cent sterile kernels, 13.0 per cent *Aspergillus*, 1.0 per cent *Penicillium*, 0.5 per cent *Chaetomium*, 2.0 per cent sterile fungi, and 2.0 per cent *Helminthosporium gramineum*. Sterile fungal mycelium developed from 8 kernels, and cultures resembling *H. gramineum* were obtained from 6 of these kernels. Barley seed inoculated with these cultures produced the following percentages of stripe-diseased plants: 27, 39, 51, 65, 0, 0, whereas the noninoculated controls were disease-free. The identification of the fungus was confirmed not only by disease symptoms but also by production of typical conidia on lesions of diseased leaves. This showed that the stripe fungus, *H. gramineum*, may remain viable in barley kernels for at least 10 years.

#### PLATING RESULTS FROM BARLEY LOTS INOCULATED WITH *FUSARIUM CULMORUM* AND *F. AVENACEUM* IN 1933

In 1933, separate plots of Oderbrucker barley were inoculated several times with conidial suspensions of *Fusarium culmorum* (W. G. Sm.) Sacc. and *F. avenaceum* (Fr.) Sacc. during and after the flowering period. The plots were covered with muslin cages, which were sprinkled with water several times each day in order to maintain a high humidity. A count from visual examination showed that each of these organisms blighted approximately 80 per cent of the kernels. The lots of barley were stored in the laboratory under dry conditions, and on November 20, 1935, random 100 kernel samples were plated. At that time, 28 months after harvest, neither *F. culmorum* nor *F. avenaceum* was found viable. Yet, at 9 months after harvest, in lots of 1932 barley grown, inoculated, and stored in the manner described above, high percentages of both organisms were found viable.

#### STABILITY OF THE EMETIC PRINCIPLE IN SCABBED BARLEY

After plating tests had been completed on the barley inoculated under the scab cages in 1931 (Tables 2 and 3), it was desired to determine whether or not the emetic principle of *Gibberella saubinetii* still remained active in these lots of grain. Small pigs were used as test animals, since only a small amount of the barley remained. The barley lot stored in the laboratory and that stored at the University-farm seed house were prepared and used sepa-

rately for drenching pigs. The samples were ground and thoroughly extracted with water at room temperature.<sup>5</sup> The extracts were then concentrated at 50° C. under reduced pressure. A water extract of healthy Oderbrucker barley grown in 1935 was prepared by the same method and used for the control.

Eight pigs of the same age, weighing between 12 and 13 pounds each, were used for the test, thus providing duplicate pigs for each extract. Each pig was given 100 c.c. of water extract from 100 grams of barley. The experiment was performed on April 9, 1936, a period of 56 months after the barley was harvested.

TABLE 5.—*Emetic activity of water extracts of scabbed barley administered to pigs. Barley inoculated under the scab cages in 1931 and tested for emetic activity on April 9, 1936 (56 months after harvest)*

Water extract of barley	Time from drenching to		Total emissions
	First emission	Last emission	
	<i>Min.</i>	<i>Min.</i>	<i>No.</i>
Scabbed barley stored in laboratory .....	11	76	17
“ “ “ “ “ “ .....	7	59	14
Control (healthy barley) .....	—	—	0
“ “ “ “ “ “ .....	—	—	0
Scabbed barley stored in University-farm seed house .....	9	68	13
Scabbed barley stored in University-farm seed house .....	11	131	14
Control (healthy barley) .....	—	—	0
“ “ “ “ “ “ .....	—	—	0

The results showing the emetic activity of these barley samples are given in table 5. Pigs drenched with water extracts of healthy barley gave no emissions. The water extract from scabbed barley stored in the laboratory as well as that from scabbed barley stored in the University-farm seed house were both highly emetic. The difference in reaction of the 2 groups of pigs to the 2 lots of scabbed barley was small and did not appear significant. Apparently, the difference in storage conditions did not appreciably affect the emetic principle. However, it was shown that both lots of scabbed barley were highly emetic at a period of 56 months after harvest and that the emetic principle remained active for at least 26 months after plating tests showed that *G. saubinetii* was not viable in any of the kernels. The stability of the emetic principle produced by this fungus in barley kernels was independent of life in the fungus.

<sup>5</sup> The author is indebted to A. D. Dickson, of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, for preparation of the water extracts and assistance in making the tests.

## DISCUSSION AND SUMMARY

The plating data of barley inoculated with *Gibberella saubinetii* indicated that viability of the fungus decreased very little during the first 9 months after harvest. The curves in figure 1 tend to follow the typical survival curve. At 9 or 10 months after harvest, a period of rapid decline began, which continued through the 18th month in barley of the 1930 crop and through the 15th month in the 1931 crop. For the most part, these curves appear to be similar to those published by Pearl (8) on the survival of *Drosophila*. They differ somewhat in that the descent of the curve at old age is more rapid for *Drosophila* than for mycelium of the scab fungus imbedded in the tissues of barley kernels.

A test of the emetic principle in scabbed barley showed that it remained active for a comparatively long period of time. The barley was inoculated with *Gibberella saubinetii* under the scab cages in 1931, and 86 per cent of the kernels was infected. A test in which pigs were drenched with water extracts of this barley showed that the emetic principle remained highly active at 56 months after the barley was harvested. At the time of the experiment, the scab fungus had been nonviable according to plating tests in all of the kernels for at least 26 months.

The plating results show that there are wide differences in the capacity of different species of fungi to remain viable in stored grain. From inoculated barley kernels, *Fusarium culmorum* and *F. avenaceum* were not plated at 28 months after harvest, while *Gibberella saubinetii* remained viable for at least 27 months. Naturally inoculated barley from Missouri Valley, Iowa, did not plate *G. saubinetii* at 20 months after harvest. An unidentified species of *Fusarium* was plated after 57 months of storage in the laboratory. *Alternaria* remained viable for 57 months in barley kernels produced in the humidity cages, and it was plated 75 months after harvest from the Missouri Valley, Iowa, sample. The *Helminthosporium* spp. producing conidia in the platings retained viability in barley kernels for as long as 51 months. *Helminthosporium gramineum* was isolated from the Missouri Valley, Iowa, sample at 75 months and from the Janesville, Wis., sample at 123 months after harvest. The identification and pathogenicity of *H. gramineum* cultures were established by inoculation experiments.

The longevity of fungi in barley kernels apparently is affected to some extent by storage conditions. *Alternaria*, *Helminthosporium*, and *Penicillium* lost their viability sooner when stored in the seed house than when stored in the laboratory. During the cold winter months, the viability of *Gibberella saubinetii* decreased less in the sample stored in the University-farm seed house than in that stored in the laboratory. The rapid drop in viability of *G. saubinetii* during the summer months possibly was caused

by higher temperature and it possibly was influenced to some extent by the moisture content of the infected kernels.

Since *Gibberella saubinetii* begins to lose its viability in barley kernels at approximately 9 or 10 months after harvest, platings made after this period cannot be considered as indicating accurately the original amount of infection.

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## THE INTERRELATION OF THE PATHOGENICITY OF A PHOMA AND A FUSARIUM ON ONIONS<sup>1</sup>

GLEN N. DAVIS AND W. J. HENDERSON<sup>2</sup>

(Accepted for publication March 29, 1937)

In 1930 a disease complex showing pink root and onion bulb-rot symptoms occurred generally in the onion-growing districts of St. Ansgar, and Clear Lake, Iowa. In some fields from 10 to 90 per cent of the plants died in the seedling stage, and approximately 30 per cent of the bulbs that matured showed dry-rot lesions of various dimensions. Another 15 per cent of the apparently healthy bulbs that matured developed fusarium bulb rot in storage. The disease complex has caused abandonment of about 200 acres of valuable onion land in Iowa, and the same is probably true of even larger sections in other States where the two diseases are known to occur.

A similar bulb rot has been reported by Selby (5), Clinton (1), Hanzawa (3), Walker and Tims (7), and Link and Bailey (4), in which the causal organisms were shown to be wound parasites. While Hanzawa (3) described the organism with which he worked as *Fusarium cepae* Hanz., Link and Bailey (4) later identified what they believed to be a similar organism as *F. zonatum* forma 1. Pink root was first reported by Taubenhaus and Mally (6) and thought to be caused by a hitherto unreported species of *Fusarium*, which he described as *F. mali* Taub. Hansen (2), however, was unable to verify the results of Taubenhaus, but was able to cause typical pink-root symptoms when he inoculated onion plants with *Phoma terrestris* Han. Although the two above-mentioned organisms (*F. zonatum* forma 1 and *P. terrestris* Han.) are reported as having approximately the same geographical distribution, the relation between them has not been demonstrated and the severe loss of seedlings in the field due to pink-root has not been reported. In view of the controversy over the cause of pink root and the lack of an adequate explanation of a wound parasite causing such a serious and widespread bulb rot, a study of the disease complex was started in 1931. The work involved studies of the causal organisms, their relationship, and methods of their control.

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<sup>2</sup> These studies were conducted in the plant pathology laboratory of the Iowa Agricultural Experiment Station.

The authors gladly take this opportunity to express their appreciation to Dr. I. E. Melhus for proposing the problem, offering timely suggestions during the course of experimentation, and reading the manuscript. They are indebted also to Dr. C. S. Reddy for many helpful suggestions, and to Mr. Sam Kennedy of Clear Lake for a tract of land and onion storage facilities.



## THE DISEASES

The symptoms of pink root and bulb rot on mature onion bulbs have been described by various investigators. However, since the progressive field symptoms from the seedling stage to the mature onion bulb have not been adequately described, the authors felt justified in including the following section on the symptoms of the two diseases.

## Pink Root

The causal agent of pink root of onions may attack the roots of the host at all stages of development from the seedling to maturity. The root tips first lose their whiteness and become a dull lead color. Usually, this condition is followed by a loss of turgidity and complete collapse of the cells of infected roots. Later the roots shrivel, change to pink, dark red, or purple, and later may become detached from the plant. Microscopic examinations disclosed that the infected root tissues were invaded by fungus mycelium frequently swollen into dark brown, knot-like bodies.

The earliest foliage symptom is the whitening of the tip of the primary leaf of the seedling, followed by a browning from the tip downward. Finally, the leaves die, become dry, and blow away. In more mature plants the first symptom is the grayish discoloration of the older cover leaves, followed by a browning, as they die, from the tip downward. Later, the younger leaves show the same symptoms as the older ones. The entire top, however, does not fall to the ground, as in the case of the seedling, but usually the upper half of the affected leaves droop, while the lower half, although dead, remains rigid and upright. A large percentage of the plants infected with the pink-root organism may not manifest any symptoms other than the discoloration of the roots. In such cases the bulb plate often continues to thicken and new roots develop.

## Bulb Rot

The first evidence of this disease is a progressive yellowing and dying back of the leaves from the tips. Sometimes, only a part of the leaf shows a yellowing and dying extending from the tip to the base. Again, the leaves may die completely within 1 or 2 weeks or survive until harvest. When the first signs of the disease appear on the leaves, necrosis has started on one side of the bulb at the upper and outer edge of the bulb plate. In every case, the roots arising from this section of the bulb plate are dead and dark red to purple. A wet rot gradually affects all the tissues of the succulent scaly leaves of the bulb from the base upward. All the roots become pink to dark red and then die. When the bulbs are attacked early by the bulb-rot organism only a mummy remains by harvest time. In other cases, where infection occurs late or fails to advance in the bulb during the growing season, it



develops in storage, leaving dry, shriveled mummies. In red onions there is commonly a greenish border in advance of the actual necrosis.

Observation of infected plants in the field and greenhouse, and of plants grown and inoculated in the greenhouse, and of bulbs in storage, shows that the description of the disease, as given by Walker and Tims (7, p. 683-684), is applicable, for the most part, to the bulb rot as it occurs in Iowa.

#### CAUSAL ORGANISMS

##### Cultural Studies

In the beginning of this work numerous isolations were made from seedlings fresh from the field and from infected bulbs that had been held in storage. Although numerous fungi and bacteria were taken from the diseased roots and bulb tissues, a *Fusarium* sp. and a *Phoma* sp. appeared consistently. The *Fusarium* could be isolated from both the infected roots and bulbs, while the *Phoma* was obtained only from roots showing the initial symptoms of pink root. The two above-mentioned organisms were identified as *F. zonatum* (Sherb.) Wr. *forma 1* Link and Bailey (4) and *P. terrestris* Hensen.

*Relation of Temperature to Growth.* The relation of temperature to growth of the two organisms was determined in the usual manner on hard potato-dextrose agar in Petri dishes placed in thermostatically regulated

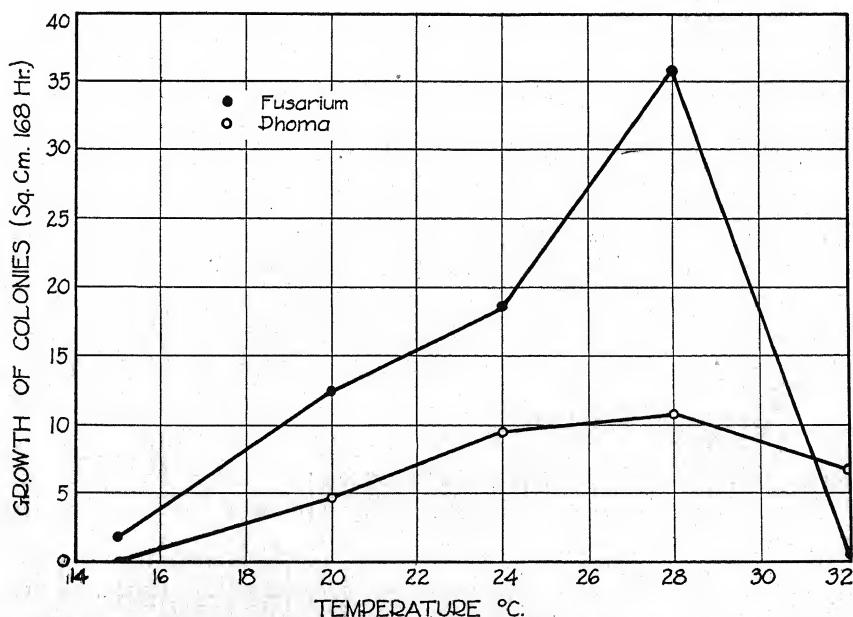


Fig. 1. Relation of temperature to growth of *Fusarium zonatum forma 1* and *Phoma terrestris*.

incubators. The area of the colonies in square centimeters after 168 hours was taken as a criterion of the growth rate. Both organisms (Fig. 1) show a gradual increase in rate of growth from 15° to 28° C., with a rapid drop above 30° C.

*Relation of Hydrogen-ion Concentration to Growth.* To study the reaction of the organisms to various concentrations of H and OH ions, potato-dextrose agar was melted and adjusted to the proper pH value immediately prior to pouring into Petri dishes. The plates were inoculated and placed in an incubator adjusted to 25° to 28° C. The results presented in figure 2

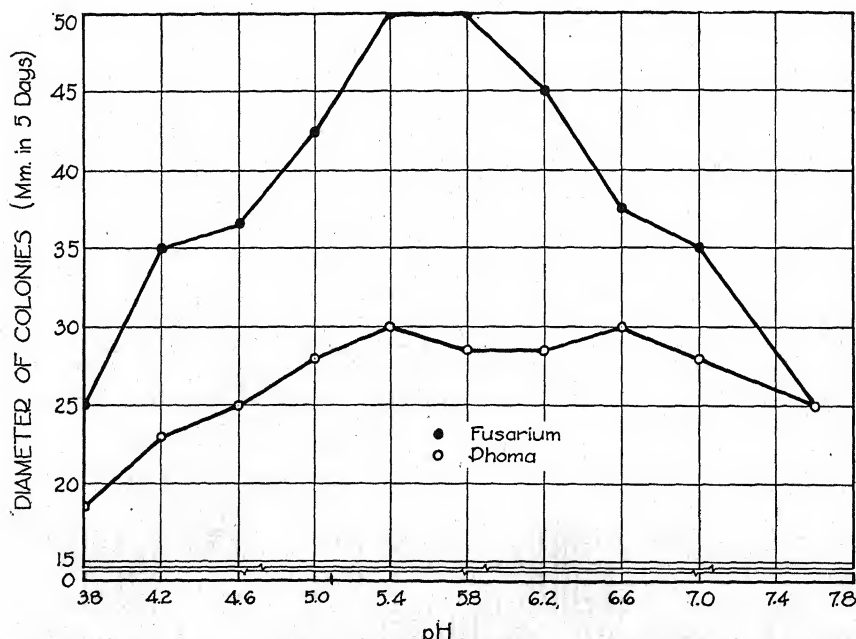


FIG. 2. Relation of hydrogen-ion concentration to growth of *Fusarium zonata forma 1* and *Phoma terrestris* at 25 to 28 degrees C.

show that both the *Phoma* and *Fusarium* grow readily over a wide pH range of 4.2 to 7.4 with the *Fusarium* making the most rapid growth throughout, although showing a tendency to be more sharply limited on the alkaline side of the scale than the *Phoma*.

#### PATHOGENICITY TESTS

##### Methods

In order to study the pathogenicity of *Phoma terrestris* and *Fusarium zonatum forma 1* to onions, mass cultures of each of the organisms were grown on a sterile barley medium. The fungus cultures were mixed singly and in combination with autoclaved peat soil in 6-inch clay pots, in all except

one case to be mentioned later. The fungus soil infestations were made as follows: In each experiment, 24 6-inch pots of autoclaved peat soil were divided into 4 groups of 6 pots each. Into each pot of soil of set 1 was mixed 6 grams of *Fusarium* mass culture; in set 2, 6 grams of *Phoma* culture, and in set 3, a mixture of 3 grams each of *Phoma* and *Fusarium*, while 6 grams of sterile barley was added to each check pot of group 4.

All onion seed and bulbs used in these experiments were immersed in (1-500) HgCl<sub>2</sub> solution for 20 minutes, a few hours prior to planting.

### Seedling Infection

A quantity of seed was planted in each pot and thinned to 50 seedlings after germination. An examination of the leaves and roots of the seedlings grown in the fusarium-infested soil showed no evidence of infection. However, all the seedlings grown in the phoma, and also in the phoma- and fusarium-mixed infested soil were dead after 1 month, and examination of the roots showed typical pink-root symptoms. The check plants in autoclaved soil remained healthy.

Isolations from diseased seedlings yielded only *Phoma* from those grown in phoma-infested soil, while roots from seedlings grown in phoma-plus-fusarium-infested soil gave isolates of both *Phoma* and *Fusarium*. The roots of the seedlings from the check and fusarium-infested soil remained disease-free, and no organisms were recovered when the roots were plated on potato-dextrose agar.

### Set Infection

Three healthy onion sets were planted in each pot of a series similar to that used in the seedling-infection experiments.

Careful examination of the plants after growing 30 days in the fusarium-infested soil showed that the roots and tops were healthy and growing normally. The plants grown in the phoma-infested soil were, however, all dead after 30 days, and definite symptoms of pink root were manifested by the roots and tops. *Phoma* was isolated from the roots of these plants, but was not obtainable from the bulb tissues. The plants grown in the fusarium- and phoma-infested soil were all dead after 30 days; and the roots were pink to dark red, indicating pink-root infection. Isolations from infected roots gave both *Phoma* and *Fusarium*. *Fusarium*, but not *Phoma*, also was isolated from the tissue above the bulb plate.

### Mature Bulb Infection

In the following experiment two mature, healthy Red Globe onion bulbs were planted in each pot. Four months from date of planting, the pots in the above experiment were emptied and a thorough examination made from root and bulb tissue of representative specimens. The bulbs grown in

fusarium-infested soil had healthy roots and tops, and plates gave negative results. The plants taken from the phoma-infested soil showed typical pink-root symptoms and were all dead at the end of the 4-month period. *Phoma*

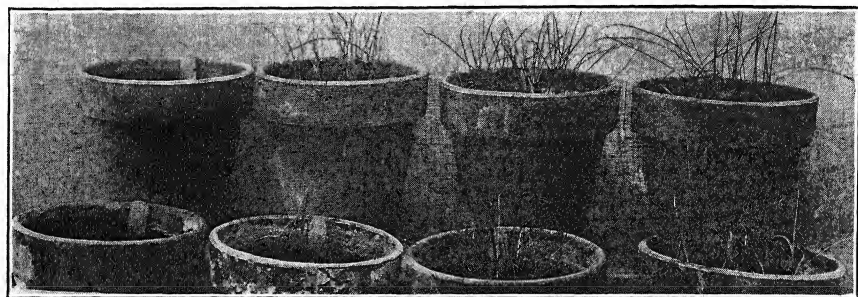


FIG. 3. Red Globe onion seedlings from seed planted in soil infested with *Phoma* (A), *Phoma* and *Fusarium* (B), and *Fusarium* (C), and in noninfested soil (D).

was isolated at will from sections of diseased roots, but was not once isolated from bulb tissue. Plants from phoma-plus-fusarium-infested soil also were dead and, upon examination, showed typical pink-root symptoms on the tops and roots. In this case, however, it was observed that the bulb plate and roots could be separated from the fleshy part of the bulb with little effort. A semi-dry rot, typical of bulb rot, was found in the basal end of the fleshy tissues. Isolations from the bulb tissue consistently gave cultures of *Fusarium*, and here, as in isolations from group 2, *Phoma* was not isolated. Sections of diseased roots, however, yielded cultures of both. The check plants in group 4 remained healthy.

An additional study with mature onion bulbs was made in which a series of soil, treated as in the foregoing experiments, was placed in pint milk bottles to facilitate observation of the roots. A mature bulb was seated in the mouth of each bottle and sealed in place with white vaseline (Figs. 4 and 5), a refinement of Hansen's (2) method.

Each of the foregoing experiments was repeated 3 times, with similar results, and the data obtained therefrom showed that *Phoma terrestris* caused the disease of onion seedlings, set plants, and mature onion plants commonly referred to as pink root of onions, thus confirming Hansen's (2) work with this organism. *Fusarium zonatum* forma 1, alone, infected the roots or bulbs of healthy onion plants only when the plants had been previously injured. This result is in agreement with that of Link and Bailey (4). However, when *P. terrestris* and *F. zonatum* forma 1 were present in the same soil, the *Phoma* made the initial invasion of the onion roots and was followed by the wound parasite *F. zonatum* forma 1. *P. terrestris* did

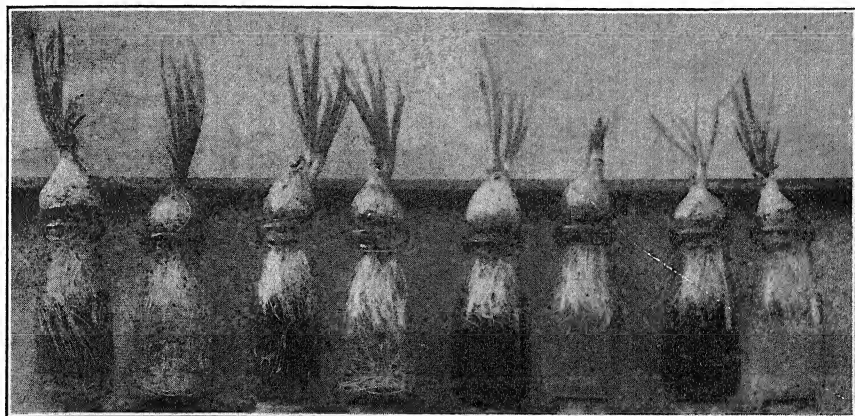


FIG. 4. Appearance of the roots of Red Globe onions when grown in (A) autoclaved soil and in soil infested with (B) *Fusarium*, (C) *Phoma*, and (D) *Phoma* plus *Fusarium*.

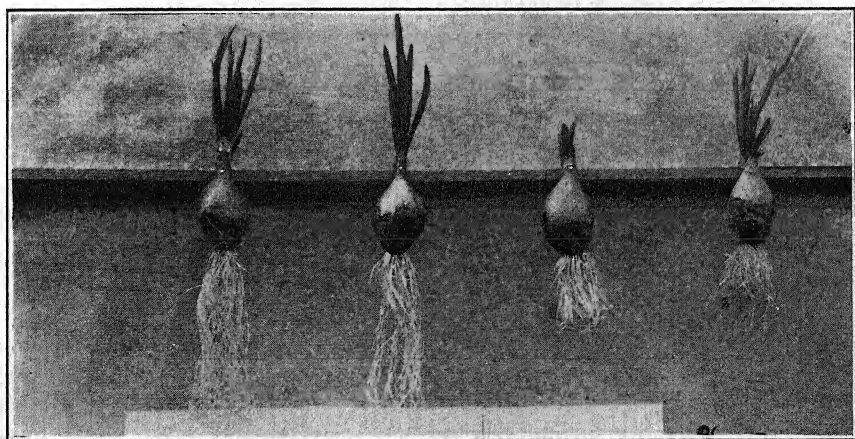


FIG. 5. Comparison of the roots of Red Globe onions from (A) autoclaved soil and from soil infested with (B) *Fusarium*, (C) *Phoma*, and (D) *Phoma* plus *Fusarium*.

not enter the fleshy portion of the bulb, but *F. zonatum* forma 1 did, probably through the vascular system, and caused bulb rot. When both organisms were present, the symptoms manifest in the roots were the same as those given for pink root or when *P. terrestris* alone was present. However, the leaves showed a combination of symptoms of both diseases and the bulbs showed the semi-dry rot, as described for bulb rot.



## CONTROL STUDIES

Inasmuch as pink root and bulb rot are caused by soil-borne organisms that persist in the soil, efforts at control were conducted for a number of years long 3 lines: (1) seed treatment, (2) soil treatment, and (3) the development of strains of onions resistant to the causal organisms.

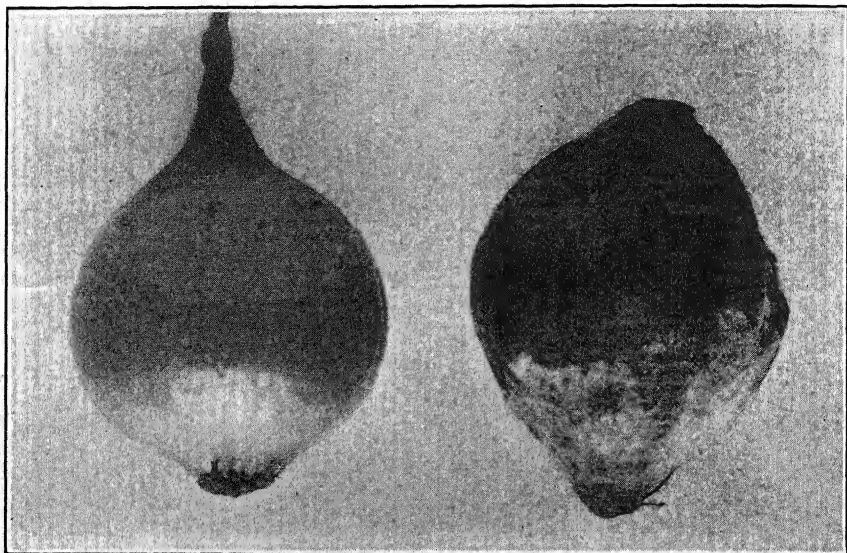


FIG. 6. Healthy onion (left) and onion infected with *Fusarium zonatum* forma 1.

It is sufficient to say that the results obtained by seed and soil treatment were very erratic, and it was apparent that such methods were of little value in controlling pink root and bulb infection.

In 1931, 35 varieties of onions were tested under field conditions. While none was immune, variable degrees of susceptibility were found, as one might expect. However, the resistance of none of the 35 varieties was considered sufficiently great to be of commercial importance.

The possibility of selecting resistant strains was considered early in the study of this disease complex. In 1930, apparently healthy Red and Yellow Globe onion bulbs were selected by Messrs. Melhus, Reddy, and Henderson from fields at Clear Lake, where 90 to 95 per cent of the plants were infected. With this selected stock as a basis, selections of bulbs have been made each year from inbred and open-pollinated strains originating with the selections made in 1930.

A review of table 1 shows only slight progress in the development of a strain of onions resistant either to pink rot or fusarium bulb rot in the open-pollinated stock. On the other hand, self pollinated selections showed dif-

TABLE 1.—*Number of bulbs with pink rot and fusarium bulb rot in self- and open-pollinated selected Red and Yellow Globe onion bulbs in summer, 1935, and winter, 1935 and 1936*

Selection	Bulbs harvested Sept. 15, 1935			No. bulbs that developed bulb rot in storage	
	Total No.	No. with pink root	No. with bulb rot	Nov. 12, 1935	Apr. 1, 1936
1 .....	37	34	4	1	0
2 .....	35	31	4	0	0
3 .....	14	11	3	0	0
4a .....	.....	.....	.....	.....	.....
5 .....	47	36	11	0	0
6 .....	22	19	3	1	0
7 .....	51	51	0	3	0
8 .....	38	26	12	1	0
9 .....	90	85	5	0	0
10 .....	43	43	3	4	3
11 .....	28	19	9	1	0
12 .....	27	25	2	0	1
13 .....	228	223	5	1	0
14 .....	42	39	3	2	0
15 .....	20	16	4	0	0
16 .....	67	42	15	0	0
17b .....	.....	.....	.....	.....	.....
18b .....	.....	.....	.....	.....	.....
19 .....	87	84	3	1	0
20b .....	.....	.....	.....	.....	.....
21 .....	83	72	11	4	0
22 .....	11	11	1	2	0
23a .....	2181	1760	406	120	46

<sup>a</sup> Yellow globe open-pollinated, other lines were selfed.

<sup>b</sup> Lines 4, 17, 18 and 20 died out in summer of 1935.

ferences in the degree of resistance. While only a single strain, No. 7, was found in which no fusarium bulb rot was evident at harvest time, 4 years of selfing and selecting have yielded several strains that showed no bulb rot in storage in 1935 and 1936. Several other strains are in hand in which the loss during storage was comparatively small. Although the 1935 and 1936 data in some cases are based on a small number of onion bulbs, other instances are shown (Strains 5, 13, and 16) in which the results are based on a larger number of bulbs, and the results are considered to be highly significant.

Less progress has been made in developing a strain of onions resistant to pink root. All inbred and open-pollinated selections showed a high percentage of pink root at harvest time, although a considerable reduction was noted in certain of the inbred selections (5, 8, 11, and 16).

#### SUMMARY

A complex of pink root and bulb rot of onions has caused the temporary abandonment of about 200 acres of valuable onion land in Iowa. Similar

conditions probably occur in other States where these diseases are known to occur.

The pink-root organism, *Phoma terrestris*, causes a severe loss of onion seedlings in infested fields, and may attack the roots of the host plant at any time during the growing season.

The causal organism of bulb rot, *Fusarium zonatum forma 1*, causes a semi-dry rot of onion bulbs in the field and in storage. It will not, however, attack the roots or bulbs, except following injury or the initial invasion by another pathogen, such as *Phoma terrestris*.

*Phoma terrestris* and *Fusarium zonatum forma 1* have the same optimum temperature (28° C.). Both organisms grow readily over a pH range of 3.8 to 7.6, with an apparent optimum of about 5.4 to 5.8. *Fusarium*, however, is apparently more sharply limited on the alkaline side of the scale than is *Phoma*.

Soil and seed treatment proved ineffective in controlling either pink root or bulb rot.

Selection and inbreeding of Red and Yellow Globe onions have resulted in the isolation of 5 strains in which the loss from bulb rot in the field and in storage was less than 5 per cent in 1935 and 1936, when the loss in the checks was 90 per cent. Little progress has been made in the development of strains of onions resistant to pink root.

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## A GREENHOUSE METHOD FOR TESTING RESISTANCE TO CURLY TOP IN SUGAR BEETS

N. J. GIDDINGS

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Breeding sugar beets for resistance to curly top requires the testing of hundreds of thousands of beet plants in order properly to evaluate the relative resistance of different individuals and strains. Most of this work is done in field plots and involves the use of natural plus artificial inoculation. It seems desirable, however, to employ an intensive greenhouse method for testing selected strains. This paper describes such a method and presents some of the results obtained through its use.

My first greenhouse tests were made in 1930 with standard 6-inch flower pots containing 4 beet plants per pot. The pots were found unsatisfactory, even when arranged in replicated groups containing one pot of each strain. Variation between individual pots, due to differences in soil, porosity of pots, watering, etc., was largely overcome by planting 4 different beet strains in one pot. But this also was unsatisfactory, especially, when several strains were being tested.

Later, some boxes were designed for use in these resistance tests. They were constructed of redwood and were  $22\frac{1}{4}$  in. long  $\times$   $5\frac{1}{2}$  in. wide, inside measurement. Some were  $4\frac{3}{4}$  inches deep, for very small plants, and others were  $6\frac{3}{4}$  inches deep, for larger plants. Holes in the bottom provided drainage, and 2 cleats across the bottom facilitated handling and better aeration. Twelve plants per box were used in most tests. They were planted in pairs and the number of strains used in any trial was such that one strain did not always occur at the same position in the box. This made it possible to expose the cultures similarly to such environmental conditions as moisture, soil, light, and heat.

Inoculations usually were made in the young 2-true-leaf stage (about 5 to 8 days after transplanting). One viruliferous beet leaf hopper was used on each plant, and allowed to remain on the plant 7 days.

Notes were taken at the end of the inoculation period, at 3- or 4-day intervals for about 2 weeks, and then at weekly intervals. Final readings were made after 5 to 6 weeks. Each diseased plant was graded for severity of symptoms, using a scale of 5 grades.

A brief description of the grading system used in the curly-top work is given below:

Plants with only slight vein clearing or occasional papillate growths on the under side are graded as 1 in severity (Fig. 1, B, C, and D).

Plants showing slight leaf curling and pronounced vein clearing or

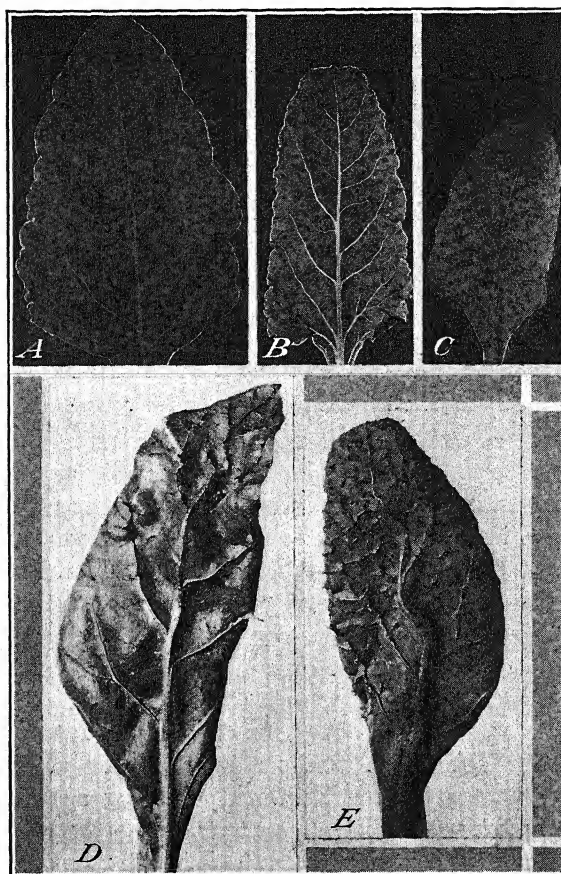


FIG. 1. A. Normal, healthy sugar-beet leaf. B. Leaf of normal shape, but showing vein clearing, most pronounced on the right side of the midvein. C. Slightly distorted leaf with vein clearing. Such vein clearing is quite characteristic of early symptoms in nearly all cases of curly top. In the very mild type of disease, there may be no other symptoms, except possibly some small papillate outgrowths from the veins on the under side of the leaf. Plants showing symptoms to this extent, or less, are classed as grade 1. D and E. Diseased sugar-beet leaves showing large and much elongated papillate growths on underside of leaf. D might be graded 1, but E would grade 2.

numerous papillae are graded as 2 in severity (Fig. 1, E, and Fig. 2, A). Plants showing pronounced curling and some dwarfing are graded as 3 (Fig. 2, B). Those showing pronounced curling and dwarfing are graded as 4 (Fig. 2, C) and those showing extreme curling and dwarfing are graded as 5 (Fig. 2, D).

From these notes on the different sugar-beet strains, it was possible to determine strain differences in percentages of infection, periods of incubation, severity of disease symptoms, and percentage of plant mortality.

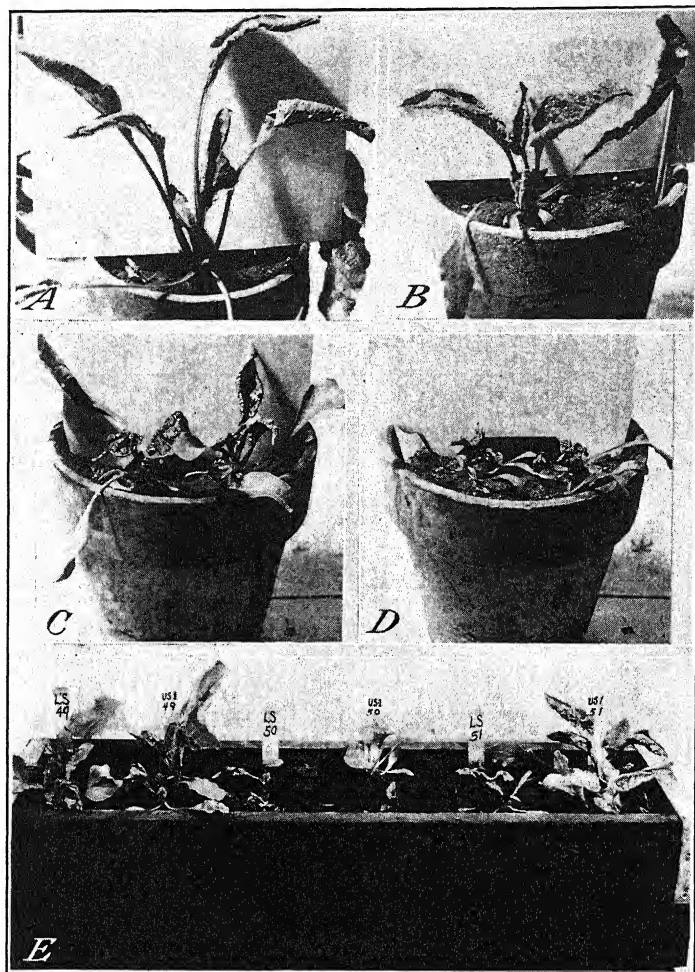


FIG. 2. A. A sugar-beet plant showing curly-top symptoms that would be graded as 2 in severity. There usually are pronounced vein clearing in the younger leaves, and numerous papillate or wart-like growths from the veins on the underside of the leaf. The leaves, frequently, but not always, show a tendency to roll or curl. B. A diseased sugar-beet plant showing pronounced leaf distortion and some dwarfing. This is classed as grade 3. C. Two sugar-beet plants much dwarfed and distorted by curly top. These symptoms are grade 4. D. Two sugar-beet plants showing extreme dwarfing and distortion. Such plants may die in a short time and the disease symptoms are recorded as 5. E. A typical plant-box test of the U. S. 1 and a susceptible strain of sugar beet. Note that the U. S. 1 plants show considerable injury from curly top, but much less than the strain marked L. S.

The first tests were made with the variety U. S. 1 and various susceptible strains, some of which were ordinary European commercial brands, while others were selections made for other purposes, without regard to resistance

to curly top. Since the reactions of these selections and of the European brands used conformed very closely, they are considered together as the susceptible group. Some of the results secured are indicated by figure 2, E, and figures 3 to 5. The data included in figure 3 cover all infected plants in 15 lots of the variety U. S. 1 and 20 lots of the susceptible group. All except 4 inoculations were made in the cotyledon stage, on or just before appearance of the first 2 true leaves. The other 4 inoculations were made on plants when in about the 4-leaf stage.

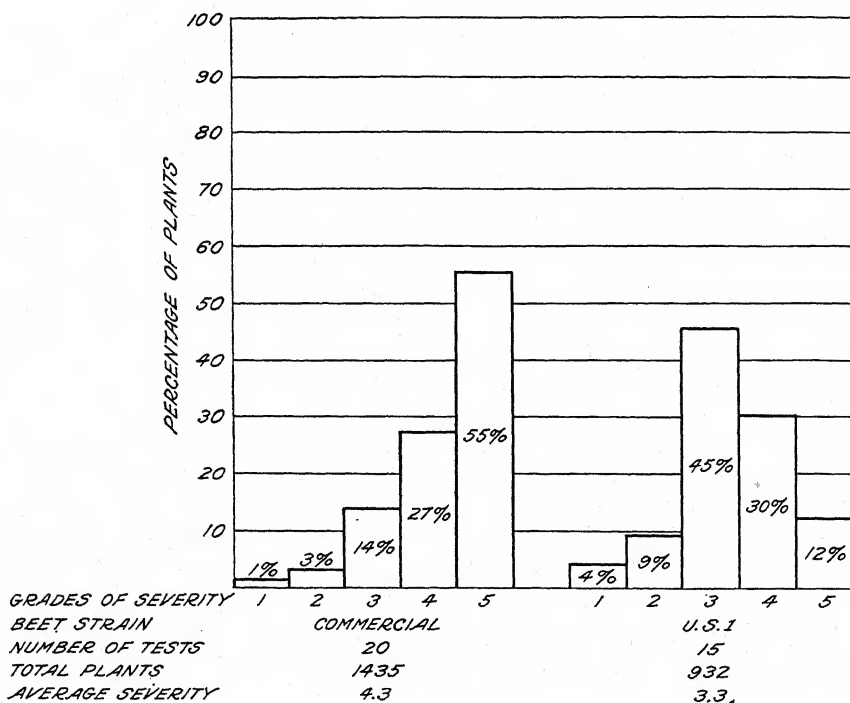


FIG. 3. Relative resistance to curly top, as indicated by severity of disease in very young plants. Summary of 15 tests of U. S. 1 and 20 tests of ordinary commercial.

It was found in this series of comparisons that out 1,831 inoculated plants of the susceptible group, 1,435 or approximately 78 per cent became infected; while out of 1,443 plants of U. S. 1, there were 932 or approximately 65 per cent infected. Since the results of every test included in this summary showed a lower percentage of plants infected in U. S. 1 than in the susceptible group, this difference is clearly significant.

On the scale of 5 degrees for severity of curly-top symptoms, the smallest difference noted in the entire series of inoculations was 0.8 and the greatest

1.5 degrees. The average of all trials shows 4.3 for the susceptible and 3.3 for U. S. 1, a difference of 1 degree.

Figure 3 indicates the distribution of infected plants into classes according to severity of symptoms. A striking difference in the relative locations of the high points is evident in this chart, with the susceptible group highest in the severely diseased class and U. S. 1 showing its resistance by a pronounced decrease in the severe class and a rise in the intermediate classes.

In every test, the curly-top symptoms appeared earlier in the susceptible group than in the U. S. 1. This indicates the greater resistance of the U. S. 1 plants and means that they were enabled to make a slightly larger growth before the disease became pronounced.

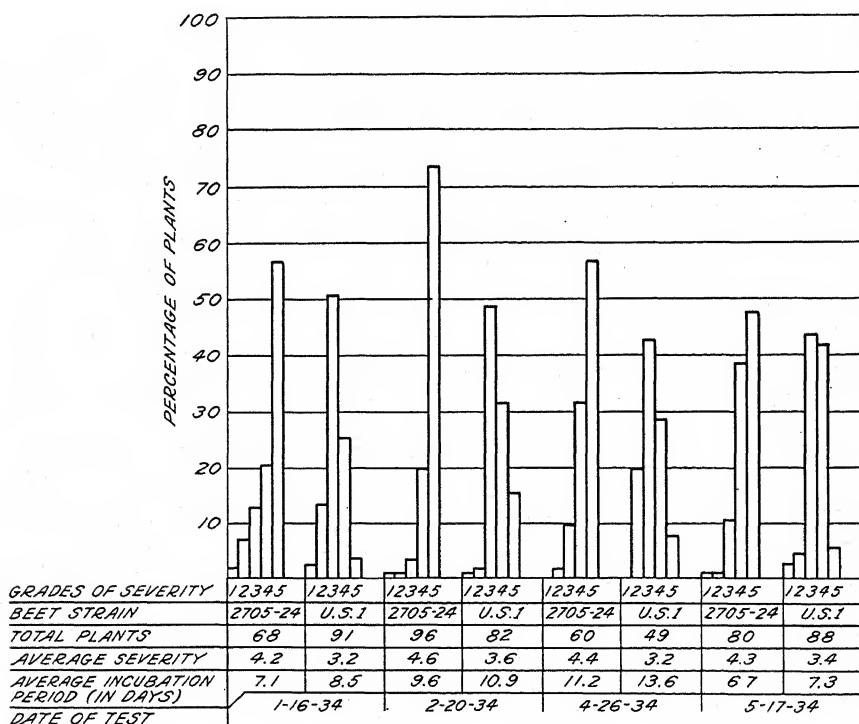


FIG. 4. Relative resistance of sugar-beet strains to curly top, as indicated by severity of disease and period of incubation in very young plants.

The U. S. 1 variety shows a significantly lower percentage of infection than the susceptible group, and the percentage of plants infected was lower for U. S. 1 in each of the tests included in these totals. The percentage of plants that died as a result of curly-top injury was also much greater in the susceptible strains than in the U. S. 1.

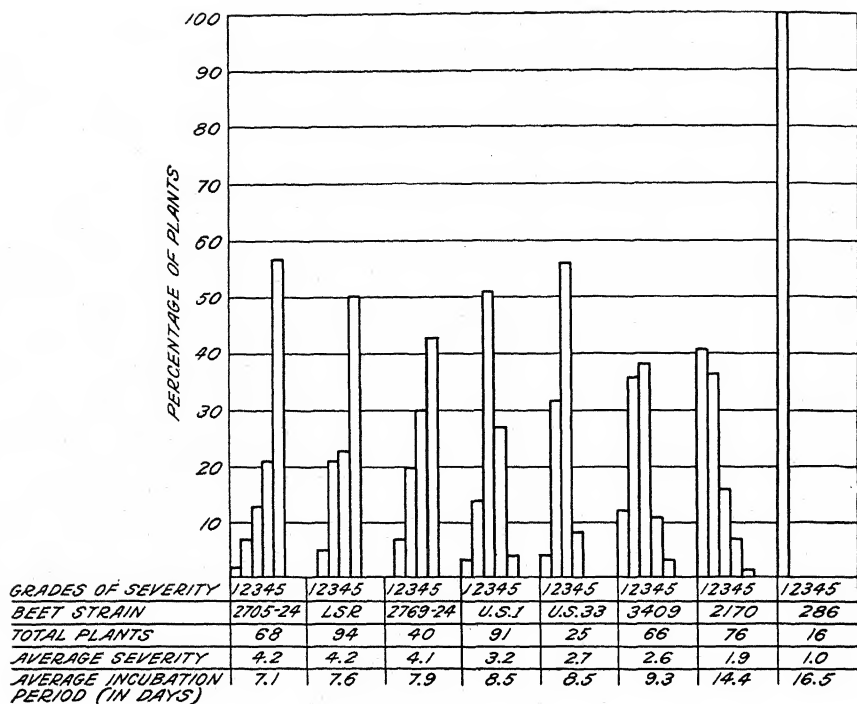


FIG. 5. Relative resistance of various strains of sugar beets to curly top, as indicated by the degree of severity of disease and periods of incubation in very young plants.

The results from several tests of U. S. 1 and a susceptible variety, designated by the breeder's number, 2705-24,<sup>1</sup> are given in figure 4. It will be noted that the smallest difference in average severity is 0.9 in the test of May 17, while the greatest difference is 1.2 in the test of April 26.

The average curly-top incubation periods for the 2 groups of plants are 10.1 days in U. S. 1 and 8.6 days in 2705-24, a difference of 1.5 in favor of U. S. 1. The least difference was 0.6 day, while the greatest was 2.4 days, the U. S. 1 showing the longest incubation period in every test.

In U. S. 1, the diseased plants that died during these experiments ranged from 0 to 10 per cent. For strain 2705-24, these values ranged from 10 to 49 per cent. Final counts were made 5 to 6 weeks after inoculation.

The histogram (Fig. 5) gives data from a typical experiment in which several strains of beets were tested. The sugar-beet strains 2705-24, Leaf Spot Resistant,<sup>2</sup> and 2769-24 are susceptibles, quite comparable with those

<sup>1</sup> 2705-24 and 2769-24 (mentioned later) designate varieties arising as a product of sugar-beet breeding conducted by W. W. Tracy, Jr. Seed of this variety came as a result of direct increase of original seed lots.

<sup>2</sup> Seed obtained from A. W. Skuderna and commonly designated as L. S. R.

regularly grown commercially, while U. S. 33,<sup>3</sup> 3409,<sup>4</sup> and 2170<sup>4</sup> are selections made from U. S. 1 for increased resistance to curly top, as well as other desirable characters. The strain 2170 was selected for especially high resistance, and the tests indicate an excellent improvement along that line. The strain 286<sup>5</sup> is a selection made by Carsner (U. S. D. A. Circular 388, 1926) primarily for disease resistance, and it is evident that a high degree of success has been attained.

It is interesting here to compare the results with readings obtained in field tests at State College, New Mexico, conducted by H. A. Elcock, as reported by Carsner.<sup>6</sup> In these tests, U. S. 1 was contrasted with 2 European brands, Pioneer and Old Type, and the curly-top reaction of each variety was determined. Each plant in the replicated tests was assigned a grade on a scale of 0 to 6, in which 0 indicated no curly top, and 6 death of the plant from the disease. The intervening grades progressively indicated slight, moderate, severe, and very severe effects. Numerous readings of individuals were averaged to obtain the variety rating. Readings were made on 3 dates in 1930 and from 4 plantings in 1931. The U. S. 1 showed distinct superiority of curly-top resistance in every case. On the basis of grade points, the least difference was 0.84 and the greatest was 1.5, in favor of U. S. 1. The average difference for the entire group was 1.17.

It would appear that greenhouse-box tests, including an adequate number of plants, give results comparable to those obtained in the field. With this greenhouse method it is possible to conduct several tests in the same time required for one field trial and between seasons when it is not possible to work in the field. The curly-top resistance grades selected should be easily approximated, permitting uniformity of results among workers.

The grouping of plants according to severity of symptoms gives important information as to degree of resistance attained, and as to uniformity of reaction among individual plants of any selection.

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<sup>3</sup> Division of Sugar Plant Investigations. New sugar-beet varieties for the curly-top area. U. S. Dept. Agr. Circ. 391. 1936.

<sup>4</sup> 3409 and 2170 are current designations for curly-top resistant strains selected by F. V. Owen at the U. S. Sugar Plant Field Station, Salt Lake City, Utah.

<sup>5</sup> 286 is a selection made because of its extreme curly-top resistance, and was later improved by F. A. Abegg at the U. S. Sugar Plant Field Station at Salt Lake City, Utah.

<sup>6</sup> Carsner, E., and others. Curly-top resistance in sugar beets and tests of the resistant variety U. S. No. 1. U. S. Dept. Agr. Tech. Bull. 360. 1933.

# STUDIES IN WOOD DECAY VI

## THE EFFECT OF ARSENIC, ZINC, AND COPPER ON THE RATE OF DECAY OF WOOD BY CERTAIN WOOD-DESTROYING FUNGI

FRANK KAUFERT AND HENRY SCHMITZ  
(Accepted for publication March 18, 1937)

### INTRODUCTION

Much experimental work has been done on the effect of iron, zinc, copper, arsenic, and other heavy metals on the growth and development of certain fungi, especially the molds. Although the results of much of this work are somewhat contradictory, it appears to be an established fact that low concentrations of at least certain of the heavy metals, for example, zinc, iron, manganese, copper, and arsenic, definitely stimulate the growth, as determined by the dry weight of the mycelium, of the fungi studied.

The published data of early workers are contradictory largely with respect to the question of whether certain heavy metals are essential for the normal growth and development of fungi. Later workers, using more highly purified salts than were available to the earlier workers, appear to have definitely established that at least some of the heavy metals are essential mineral nutrients.

For example, Roberg (6) in a comparatively recent study has confirmed the results of a number of other workers, that iron and zinc not only stimulate the growth of *Aspergillus niger*, *A. fumigatus*, and *A. oryzae*, but that these elements are essential to their normal growth and development. Roberg also showed that, despite the essential nature of zinc to mold fungi, it is toxic to them in small quantities if adequate amounts of iron are not present. Apparently there is an antagonistic effect between iron and zinc—the iron counteracting the toxic effects of the zinc. Low concentrations of copper, also, were found by Roberg to stimulate the growth of the fungi used in his investigations.

The stimulating effects of not only zinc and copper, but also of manganese, were demonstrated by McHargue and Calfee (5). The optimum concentrations of these metals in nutrient solution were found to be low, and, according to these workers, slightly larger quantities were toxic. The maximum growth of the fungi used occurred at a concentration of 5 parts copper per million of nutrient solution. With each increase in copper content above this amount a decrease occurred in the dry weight of the fungus. In the zinc series, the greatest dry weight of fungus also was produced in the presence of 5 parts zinc per million. Here again, the dry weight of fungus decreased with increases in the concentration of zinc to 20 parts per million. Greater concentrations of zinc gave inconsistent results.



McHargue and Calfee also determined the optimum concentrations of copper sulphate and zinc chloride on the growth of *Aspergillus niger* on silica gel. These were found to be 5 parts per million for copper and 1 part per million for zinc.

There appears to be some question concerning McHargue and Calfee's interpretation of their data with respect to the toxic effects of copper and zinc. Although concentrations of copper sulphate and zinc chloride greater than 5 parts per million in nutrient solution did not result in so much growth as occurred in the cultures containing 5 parts per million of these salts, it appears incorrect to regard such concentrations as exerting toxic effects, in the usual sense, because the dry weights of fungus produced in all cases up to 30 parts per million (the highest concentration used) were greater than that produced in the controls.

The stimulating effects of toxic materials have been studied in detail by Bateman (1), who believes that the conception of two opposed reactions can easily be applied to changes in concentration on the welfare of living organisms, if it is assumed that both reactions are present at all concentrations of the reacting material. If one of these reactions is beneficial and the other detrimental to the well-being of the organism, the one that has the greatest effect at any particular concentration will be the one whose influence is more apparent at that particular concentration. If the two reactions are equal at some concentration, there will be no apparent effect. At no concentration can either effect be measured alone, because of the opposing effect.

The biological effects of arsenic on fungi have been studied by numerous workers. The more important literature on this subject has been reviewed by Thom and Raper (8), who at the same time reported their own observations on the arsenic tolerance of a large number of strains of several species of *Aspergillus* and of several other fungi. Thom and Raper conclude, as the result of their work, that "arsenic fungi" are much more numerous than was previously supposed and that arsenic-tolerant forms include many species that do not decompose arsenic compounds with the evolution of gas.

Wood has been used as a substratum for testing the toxicity of numerous chemicals to wood-destroying fungi, but, so far as the writers are aware, experimental work to determine the growth stimulating effects of salts of heavy metals on fungi has been confined to nutrient culture solutions or to nutrient agar or silica gel.

Because of the fact that zinc, arsenic, and copper salts are all used as wood preservatives, it is of considerable interest and importance to determine whether or not the presence of small amounts of these salts actually increase the rate of decay of wood by wood-destroying fungi.

## MATERIALS AND METHODS

All of these tests were made with the sapwood sawdust of Norway pine, *Pinus resinosa* Ait. The sawdust was made from green logs with a power saw. Before using, the sawdust was air-dried and thoroughly mixed to assure uniformity of the samples. After an equilibrium moisture content was reached, 33.0-gram samples of the air-dried sawdust were weighed in 1-pt. square Mason jars fitted with metal screw caps from which the cardboard seals had been removed.

The moisture content of the air-dried sawdust was determined by drying 33.0-gram samples to constant weight at 104° C. At the time the series to which arsenic trioxide was added was prepared, the air-dried sawdust contained 7.27 per cent moisture; at the time the series to which zinc chloride and copper sulphate were added, it contained 9.39 per cent moisture on an air-dry basis. Because the sawdust was continually mixed, there was little variation in moisture content of the samples taken at any one time. Thus the oven-dry weight of the sawdust in each jar was 30.6 g. for the tests with arsenic trioxide, and 29.9 g. for the test with zinc chloride and copper sulphate.

U. S. P. arsenic trioxide (acid arsenous) and reagent zinc chloride, and copper sulphate were used as sources of arsenic, zinc, and copper. Stock solutions of each of these compounds were prepared by dissolving them in hot distilled water. The following concentrations of arsenic trioxide were prepared: 50, 100, 200, 400, 800, and 1000 parts per million of water. The concentrations of zinc chloride prepared were: 100, 200, 400, 800, and 1600 parts per million of water; and of copper sulphate, 100, 200, 400, 800, 1600, and 3200 parts per million of water.

Sixty cc. of the arsenic trioxide solutions were added to the sawdust in the culture jars. Since the air-dried sawdust contained about 7.8 per cent moisture on an oven-dry basis, the moisture content of the sawdust after the arsenic solutions were added was about 204 per cent. Previous tests had shown that satisfactory decay could be obtained at this moisture content, although the rate of decay was somewhat more rapid at higher moisture contents. Because preliminary tests had been made with 5, 10, 20, 50, and 100 parts of arsenic trioxide per million of water, and the sawdust in these tests had a moisture content of slightly over 200.0 per cent, it was considered advantageous to use the same moisture content in the arsenic series, in order that the results might be comparable.

In the tests with zinc chloride and copper sulphate, 90 cc. of the solutions were added to the sawdust in each culture jar. Because the air-dried sawdust in these tests contained about 10.3 per cent moisture on an air-dry basis, the moisture content of the sawdust after the zinc chloride and copper sulphate solutions were added was about 311 per cent, which previous tests

had shown to be about the optimum for decay of the sawdust of Norway pine sapwood.

In the discussion of the experimental results the various culture series are referred to as containing a certain number of parts per million of the various metals. For example, a certain series of cultures, for the sake of brevity, is said to contain 50 parts of arsenic per million. Actually, this particular culture series contained 50 parts arsenic trioxide per million in the 60 cc. water added to 30.6 grams of sawdust. Furthermore, no correction was made for the water of crystallization in the copper sulphate. Hence, no direct comparisons can be made from the data presented concerning the relative toxicity of arsenic, zinc, and copper. Had this been the objective of the experiment, molar solutions would have been used. From the point of view of application, however, it seemed advisable to determine the percentage concentrations of the salts that stimulated the growth of the fungi.

Distilled water only was added to the sawdust of the control jars, 60 cc. to the control jars of the arsenic series, and 90 cc. to the control jars of the zinc chloride and copper sulphate series.

The culture jars were sterilized in an autoclave at 10 pounds' pressure for 30 minutes, and when cool the jars were planted with isolates of several fungi. Four fungi, *Lenzites trabea* Pers., *Lentinus lepideus* Fr., *Trametes serialis* Fr., and *Polyporus anceps* Pk. were used in the tests with arsenic trioxide, and two fungi, *L. trabea* and *L. lepideus* were used in the tests with zinc chloride and copper sulphate. In every case 12 cultures were prepared for each fungus at each of the concentrations given above.

After planting, the culture jars were incubated at about 25° C. for 3½ months. At the end of this period, the culture jars and contents were dried to constant weight at 104° C. to determine the loss in weight due to decay. The difference between the original and final oven-dry weights of the sawdust in the culture jars was taken as a measure of the rate of decay. The standard error of the mean of each series was computed. The significance of the differences between the controls and the cultures to which arsenic trioxide, zinc chloride, and copper sulphate were added, was determined by "Student's" *t* test.

#### RESULTS AND DISCUSSION

The effect of different concentrations of arsenic trioxide, zinc chloride, and copper sulphate on the rate of decay of sawdust of Norway pine sapwood by *Lenzites trabea*, *Lentinus lepideus*, *Trametes serialis*, and *Polyporus anceps* is shown in table 1 and figure 1.

*Effects of Arsenic Trioxide.* The presence of 50 or 100 parts per million of arsenic trioxide clearly increases the rate of decay of Norway pine sapwood by *Lenzites trabea* and by *Lentinus lepideus*. The addition of 50 and 100 p.p.m. of arsenic trioxide stimulated the growth of *Lenzites trabea*. Two

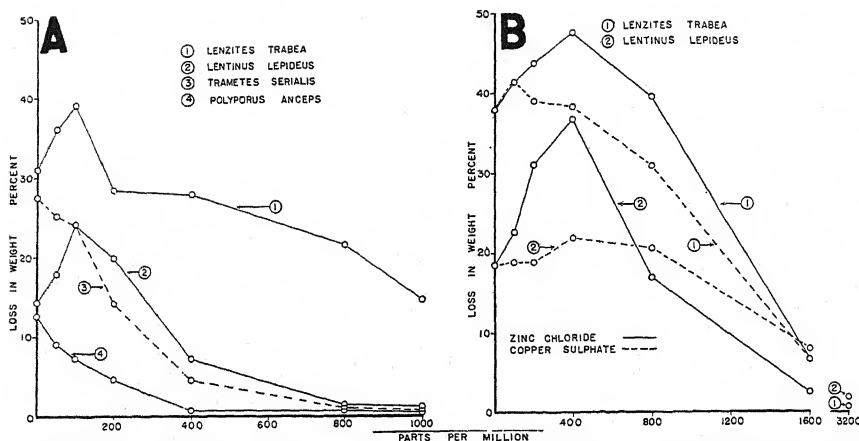


FIG. 1. The effect of different concentrations on the rate of decay of sawdust of Norway pine sapwood by wood-destroying fungi. A. Arsenic trioxide. B. Zinc chloride and copper sulphate.

TABLE 1.—The influence of different concentrations of arsenic trioxide, zinc chloride, and copper sulphate on the rate of decay of Norway pine sapwood sawdust by wood-destroying fungi

Concentration pts. per m.	Average loss in weight			
	<i>Lenzites trabea</i>	<i>Lentinus lepideus</i>	<i>Trametes serialis</i>	<i>Polyporus anceps</i>
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
<b>Arsenic trioxide</b>				
None .....	31.0 ± 0.87	14.3 ± 0.26	27.5 ± 0.65	12.7 ± 0.33
50 .....	36.1 ± 0.83	17.9 ± 0.44	25.2 ± 0.82	9.1 ± 0.50
100 .....	39.1 ± 0.70	24.1 ± 0.60	24.3 ± 0.62	7.3 ± 0.32
200 .....	28.4 ± 0.49	19.9 ± 0.79	14.2 ± 0.84	4.6 ± 0.12
400 .....	27.8 ± 0.53	7.2 ± 0.65	4.5 ± 0.62	0.6 ± 0.16
800 .....	21.5 ± 0.68	1.4 ± 0.30	1.0 ± 0.22	0.6 ± 0.12
1000 .....	14.6 ± 0.86	1.2 ± 0.25	0.6 ± 0.14	0.4 ± 0.10
<b>Zinc chloride</b>				
None .....	37.9 ± 2.82	18.5 ± 0.33		
100 .....	41.3 ± 2.50	22.6 ± 0.41		
200 .....	43.7 ± 3.05	31.0 ± 0.25		
400 .....	47.5 ± 0.66	36.7 ± 1.07		
800 .....	39.4 ± 1.73	16.9 ± 1.26		
1600 .....	6.6 ± 1.14	2.5 ± 0.99		
<b>Copper sulphate</b>				
None .....	37.9 ± 2.82	18.5 ± 0.33		
100 .....	41.5 ± 1.05	18.8 ± 0.48		
200 .....	39.0 ± 1.26	18.8 ± 0.25		
400 .....	38.3 ± 2.56	21.8 ± 0.75		
800 .....	30.8 ± 3.38	20.5 ± 1.80		
1000 .....	6.6 ± 2.29	8.0 ± 1.71		
3200 .....	0.7 ± 0.26	1.8 ± 0.19		

hundred or more p.p.m. resulted in less loss in weight than in the controls. However, *Lenzites trabea* was far more resistant to the action of arsenic trioxide than *Lentinus lepideus*. Even when the concentration of arsenic reached 1000 parts per million, *Lenzites trabea* caused about 50 per cent of the loss in weight observed in the control cultures. On the other hand, the presence of 1000 parts of arsenic trioxide per million almost completely inhibited the decay of Norway pine sapwood by *Lentinus lepideus*.

*Lenzites trabea* has long been suspected of being "arsenic tolerant." Despite the almost complete absence of published data on this point, it has been frequently observed growing in wood treated with arsenic compounds. In all the cultures of this fungus, a characteristic garlic odor was plainly evident. Unless the physiology of the wood-destroying fungi is different from that of the molds with respect to their action on arsenicals, this odor was due to the presence of trimethylarsine (2).

The rate of decay of Norway pine sapwood by *Trametes serialis* and *Polyporus anceps* was retarded even by the presence of 50 parts of arsenic trioxide per million. Increasing amounts of arsenic still further retarded the rate of decay induced by these fungi. *Trametes serialis* was somewhat more resistant to arsenic than was *Polyporus anceps*. Even at a concentration of 400 parts per million, the growth of *Polyporus anceps* was inhibited almost completely.

The toxicity of various arsenic compounds to wood-destroying fungi has been determined by Falck (4) and by Curtin and Thordarson (3). Falck, for example, found that a concentration of 0.01–0.05 per cent of sodium meta-arsenite, on the basis of the dry weight of the wood, is sufficient to inhibit the growth of wood-destroying fungi. The amount of arsenic trioxide in 60 cc. of solution containing 100 parts per million is equivalent to approximately 0.02 per cent based on the oven-dry weight of the sawdust. At this concentration the maximum rate of decay by *Lenzites trabea* and *Lentinus lepideus* occurred.

*Effects of Zinc Chloride.* When the amount of zinc chloride in the cultures increased from 100 parts to 400 parts per million, the rate of decay of Norway pine sapwood by *Lenzites trabea* and *Lentinus lepideus* increased, reaching a maximum at the latter concentration. Although the differences in the percentage loss in weight of the control cultures of *Lenzites trabea* and those observed in the presence of 100 parts and 200 parts of zinc chloride per million were not statistically significant, the value observed at the 400 parts per million concentration was highly significant.

In the case of *Lentinus lepideus*, all of the increases in rate of decay up to the 400 parts per million concentration were statistically significant. It is clear that the presence of zinc chloride up to 400 parts per million definitely stimulates the rate of decay of Norway pine sapwood by *Lenzites*

*trabea* and by *Lentinus lepideus*. Concentration above 400 parts zinc chloride per million caused smaller increases in the rate of decay of Norway pine sapwood by these fungi, and, even in the presence of 800 parts zinc chloride per million, the amount of decay caused by each of these fungi is about equal to that observed in the control cultures. At a concentration of 1600 parts zinc chloride per million, appreciable losses in weight still were observed.

It appears from the data presented that it requires a considerably higher concentration of zinc chloride in wood to stimulate the rate of growth of wood-destroying fungi than it does to stimulate the growth of molds growing in nutrient culture solutions or on nutrient agar. McHargue and Calfee (5), for example, obtained the greatest weight of *Aspergillus niger* in the presence of 5 parts zinc chloride per million. In our work the greatest amount of decay caused by both *Lenzites trabea* and *Lentinus lepideus* occurred in the presence of 400 parts zinc chloride per million. However, no direct comparisons can be made because of the fact that the 90 cc. of distilled water containing the zinc was added to 29.9 grams of oven-dry sawdust, and there is considerable basis for the assumption that at least part of the zinc is adsorbed by the wood substance and rendered more or less ineffective insofar as exerting toxic effects are concerned.

*Effects of Copper Sulphate.* The data for copper sulphate given in table 1 and shown graphically in figure 1 are inconclusive. Although the loss in weight of Norway pine sapwood caused by *Lenzites trabea* in the presence of 100 parts copper sulphate per million is somewhat above that of the controls, the difference is not statistically significant. The influence of lower concentrations of copper sulphate on the rate of decay caused by this fungus was not determined, but it is barely possible that if the influence of concentrations of copper sulphate lower than 100 parts per million had been determined, a definite stimulation in the rate of decay might have been observed.

In the case of *Lentinus lepideus*, the addition of up to 800 parts copper sulphate per million did not have a marked influence on the rate of decay. The addition of 400 and 800 parts copper sulphate per million caused an apparent increase in the rate of decay, but the value for the former concentration only is statistically significant.

In general, *Lentinus lepideus* appears to be more resistant to the toxic properties of copper than does *Lenzites trabea*. A marked decrease in the rate of decay of Norway pine sapwood by *L. trabea* was noted for concentrations above 400 parts per million, and at a concentration of 3200 parts per million the growth of the fungus appears to be inhibited. The rate of decay of Norway pine sapwood by *Lentinus lepideus*, on the other hand, was not decreased until a concentration above 800 parts per million was reached. At a concentration of 1600 parts per million, *L. lepideus* caused a greater

loss in weight than *Lenzites trabea*, despite the fact that the control cultures of *Lentinus lepideus* lost only about one-half the weight lost by those of *Lenzites trabea*.

Steinberg (7) has recently shown that the optimum heavy metal concentration varies with the acidity of the solution and that alkalinity (pH 8.0 or higher) causes a marked increase in the apparent optima of heavy metals (iron, copper, zinc, and manganese) for growth, since they serve both as nutrients and chemically to increase acidity. Because of the fact that aqueous extracts of wood are rather acid, it should follow, if no other factors are operative, that optimum concentrations of these metals when added to wood should be low. However, this was not always found to be the case. Adsorption and inactivation of the metals by the cell-wall material and extractives in the wood may be a partial answer to this apparent discrepancy.

#### SUMMARY

A study has been made of the effect of arsenic, zinc, and copper on the rate of decay of Norway pine sapwood sawdust by certain wood-destroying fungi. The addition of low concentrations of arsenic trioxide to Norway pine sawdust appears definitely to stimulate its decay by *Lenzites trabea* and by *Lentinus lepideus* while even the lowest concentration tested, namely: 50 parts per million appeared to be toxic to *Trametes serialis* and *Polyporus anceps*.

Zinc chloride at concentrations of from 100 to 400 parts per million appeared to increase the rate of decay of Norway pine sapwood sawdust by *Lenzites trabea* and *Lentinus lepideus*. Concentrations above 400 parts per million appeared to be definitely toxic to both fungi.

The results with copper sulphate are inconclusive insofar as showing a significant increase in the rate of decay of Norway pine sapwood by *Lenzites trabea* and *Lentinus lepideus* is concerned. It appears that *Lentinus lepideus* is considerably more resistant to copper sulphate than *Lenzites trabea*, but the latter fungus is far more resistant to arsenic trioxide than the former.

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## CEPHALOSPORIUM CANKER OF BALSAM FIR<sup>1</sup>

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(Accepted for publication March 12, 1937)

This canker was first found on *Abies balsamea* (L.) Mill. in 1933 by F. H. Kaufert and L. W. Orr, at Itasca Park, Minnesota. Later, the same year, Kaufert found it in the Chippewa National Forest, and in August, 1935, he found it to be rather abundant at Half Way, Minnesota, in the Superior National Forest. In June, 1935, a specimen of balsam fir affected with this canker was sent to the author by Mr. M. J. O'Connell, from Smith Lake Camp, Hayward, Wisconsin. In 1936 Kaufert found numerous old cankers on balsam near Hayward, Wisconsin, but few new ones. In August, 1936, the author examined about 1,000 balsam fir trees in Itasca Park and found only one diseased. Undoubtedly, the disease is distributed throughout much of the range of balsam fir in Minnesota, and probably in Wisconsin, and the fairly heavy infection found at Half Way in 1935 indicates that in an environment favoring its spread and development it may be of some economic importance.

### DESCRIPTION OF THE CANKER

The cankered area usually is irregularly oval or elliptical, with the long axis extending up and down the tree. Frequently, but not always, the cankers appear to originate at branch stubs. The dead bark is sunken slightly and cracked at the border of the canker. Resin is exuded from broken blisters on the face of the canker and runs down the bark in streaks, this being the most obvious outward indication of the presence of a canker. The specimen illustrated in figure 1A, is scarcely typical, because, until the bark is removed, the canker is not, ordinarily, sufficiently apparent to show up at all in a photograph. Naturally, resin streaks on the bark are not infallible indicators of the presence of cankers, since the same symptom may be produced by other causes.

The diseased inner bark is brown, and often the extent of the canker can be determined only by cutting away the outer bark. There is no apparent gradual transition zone between the healthy, white and the diseased, brown inner bark. This brown discoloration sometimes extends into the wood

<sup>1</sup> Paper No. 1479, Scientific Journal Series, Minnesota Agricultural Experiment Station.



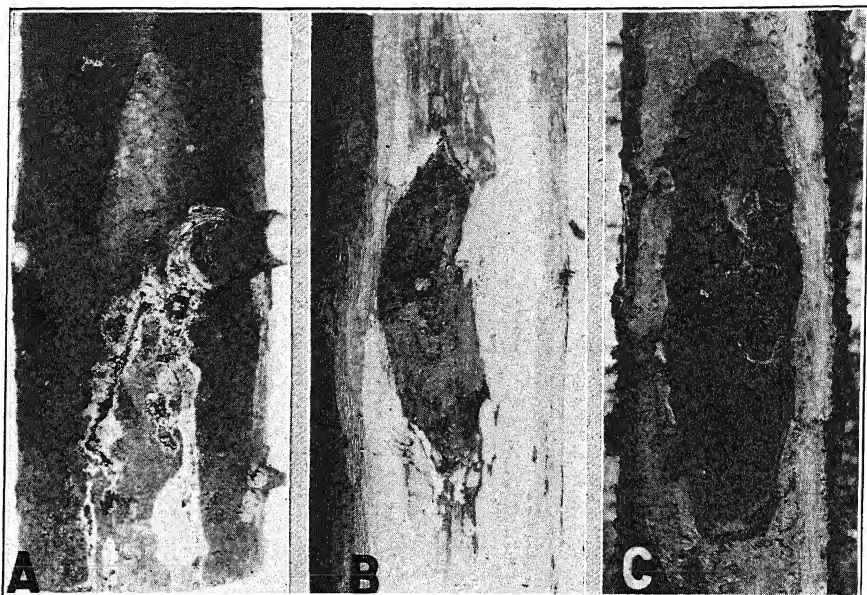


FIG. 1. A. Canker from Hayward, Wis. Note origin at branch stub and resin streaks. B. Canker from Half Way, Minn. Outer bark removed from left side, outer ring of sapwood removed from ends and right side. C. Canker from an inoculated wound. Tree 5 inches in diameter.

a distance of one or two annual rings, and may extend farther longitudinally and tangentially in the wood than it does in the bark, indicating either that the fungus is progressing more rapidly in the wood than in the bark, or that the bark is healing over. From present evidence the former explanation seems more probable. The appearance of the canker when the outer bark is cut off is shown in figure 1, B. The first annual ring of wood has been removed from the right side and ends of the canker to show the extent of the discoloration in the wood.

No cankers were found that appeared to be more than 3 or 4 years old; apparently, because the cankers either progress rapidly enough to kill the trees in a relatively few years or cease to enlarge and are overgrown by the bark. Cankers have been found most commonly on suppressed trees 3 to 5 inches in diameter, and none have been found on trees over 8 inches in diameter; it may be that the disease is favored by suppression.

#### INOCULATIONS

Kaufert isolated a fungus from the diseased specimen he found at Itasca in 1933, but did not determine its identity. The author isolated a similar fungus from specimens from Hayward, Wisconsin, and Half Way, Minnesota,

in 1935. The culture obtained from the specimen from Hayward was inoculated into 3 apparently healthy balsam fir trees, from 3 to 5 inches in diameter, at Itasca Park, in August, 1935. The trees were inoculated by placing pieces of the culture on the stubs of dead branches  $\frac{1}{4}$  to  $\frac{1}{2}$  inch in diameter, cut off flush with the trunk, and in wounds made in the bark with a knife. Twenty inoculations were made in the 3 trees, and about 10 check wounds were made. The noninoculated branch stubs also served as checks. Cankers developed around 15 of the inoculated branch stubs and wounds, and the noninoculated checks remained healthy.

When the trees were examined in August, 1936, the largest canker was about 12 inches long, and extended about  $\frac{1}{4}$  the way around the 5-inch tree. This canker, with the outer bark removed, is shown in figure 1, C. The largest canker that developed from an inoculated branch stub was about 5 inches long. In general, the cankers that developed around the inoculated branch stubs were much smaller than those from the inoculated wounds. The smallest tree, 3 inches in diameter, was practically girdled by two cankers that had developed from inoculations on opposite sides. These trees were removed from the forest immediately after they had been examined in August, 1936. The fungus was reisolated from the cankers that had developed around the inoculated wounds, and these cultures were used to inoculate a larger number of trees.

If the rate of growth on these inoculated trees is typical, it is obvious that few old cankers would be found, since the trees would be killed within a few years after they became infected. Such rapid parasitic invasion of the bark of healthy trees is unusual when compared with other canker-causing fungi native to this region, since, in the inoculated trees, the period when temperature permitted growth, of the fungus could hardly have exceeded 6 months. No evidence has been obtained on the rate of growth for more than one year, so it is quite impossible to say whether or not this rapid growth will be maintained.

#### THE CAUSAL ORGANISM

The canker is caused by a species of *Cephalosporium*; the author has not been able to find any reference to this fungus on balsam fir, and it may be a new species. According to Saccardo<sup>2</sup> (p. 58) *Cephalosporium album* is found on dead branches of pine, but his description of *C. album* is so general that one can not compare it accurately with the fungus found by the author on balsam fir; hence the author considers it desirable to study this fungus further before deciding finally upon its identity. On malt agar at temperatures from 20° to 30° C. it produces a faintly zonate culture of white, fluffy, aerial mycelium, the aerial hyphae extending 1 to 2 mm. above the surface of the agar. At lower temperatures the growth is more scanty and the mycelium

<sup>2</sup> Saccardo, P. A. *Sylloge fungorum*, v. 4. Padua. 1886.

more appressed. Preliminary temperature studies indicate that it will not grow at 0° and 35° C., grows slowly at 15° C., and most rapidly at 27° to 30° C. Conidia are produced in abundance in 3 to 4 days. On hanging-drop cultures the conidiophores are 10 to 90  $\mu$ , mostly 20 to 40  $\mu$ , long, and arise vertical to the hyphae on which they are borne. Some of the conidiophores have one to four short, secondary conidiophores arising vertically from them, but most of them are unbranched. The conidiophores taper to a slender tip, on which 1 to 3 conidia are borne simultaneously, either on short sterigmata

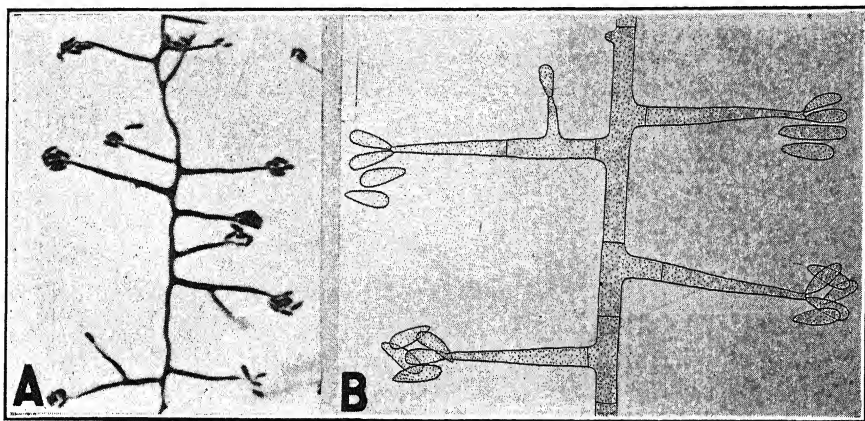


FIG. 2. The causal fungus. A. Photomicrograph. B. Drawing of conidiophores and conidia.

or directly on the conidiophores. Each sterigma continues to produce conidia, and these remain clustered in a roughly spherical head about the tip of the conidiophore. A mature head may contain 5 to 20 conidia, the average being in the neighborhood of 7 to 10. The conidia are elongate oval, or pointed at the base, and sometimes flattened on one side. Fifty spores from a malt-agar test-tube culture were measured, and they averaged 4.3  $\mu$ , and ranged from 2.8 to 5.7  $\mu$ , in length. Conidiophores and conidia from a hanging-drop culture, 5 days old, on malt agar, are shown in figure 2.

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# MODIFICATIONS OF CELL STRUCTURE IN "HALO WILDFIRE" AND "EPIDEMIC WILDFIRE"

JEAN DUFRÉNOY

(Accepted for publication Oct. 15, 1936)

As the results of Clayton's observations<sup>1</sup> indicate, the entire problem of epidemic wildfire may be reconsidered. To take, for instance, the situation in southwestern France: In 1931 *Bacterium tabacum* was isolated in pure culture from halo-wildfire lesions collected May 20 on leaves of seedlings in various seedbeds; halo wildfire was reported in various fields in July, developing into epidemic wildfire after the storms and heavy rains of August 1-2, 6-7, and 15-16 (65 mm. (approx. 2.5 in.) of rainfall).



FIG. 1. Tangential section through spongy parenchyma in the halo surrounding a lesion of *Bact. tabacum* (killed with the Némec fluid). ap, agglutinated plastids; l, oil droplets in plastids; p, plastids; pr, proteic granules in disintegrating plastids; t, flocculated vacuolar solution containing an abundance of phenolic compounds; a, starch grains within amyloplasts.

<sup>1</sup> Clayton, E. E. Toxin produced by *Bacterium tabacum* and its relation to host range. Jour. Agr. Res. [U.S.] 48: 411-426. 1934.

In 1932 lesions were observed in seedbeds about May 15; a succession of storms, some of them accompanied by hail from May 21 to June 6, resulted in widespread infection of late seedlings that had escaped earlier infection; but it was only after the storm of July 15 (45 mm. rainfall) that epidemic wildfire broke out.

In 1936 halo wildfire was observed in many seedbeds, but, although July was cold and rainy, the weather, favorable for the spread of halo lesions, was overcast and the relative humidity was higher than 73 per cent during the earlier part of August, no epidemic wildfire developed, except in a few fields that had been hit by hail, as no storms were generally experienced.

In 1937 halo lesions appeared on May 3 around necrotic spots where large raindrops had hit seedling leaves during a shower, the day before.

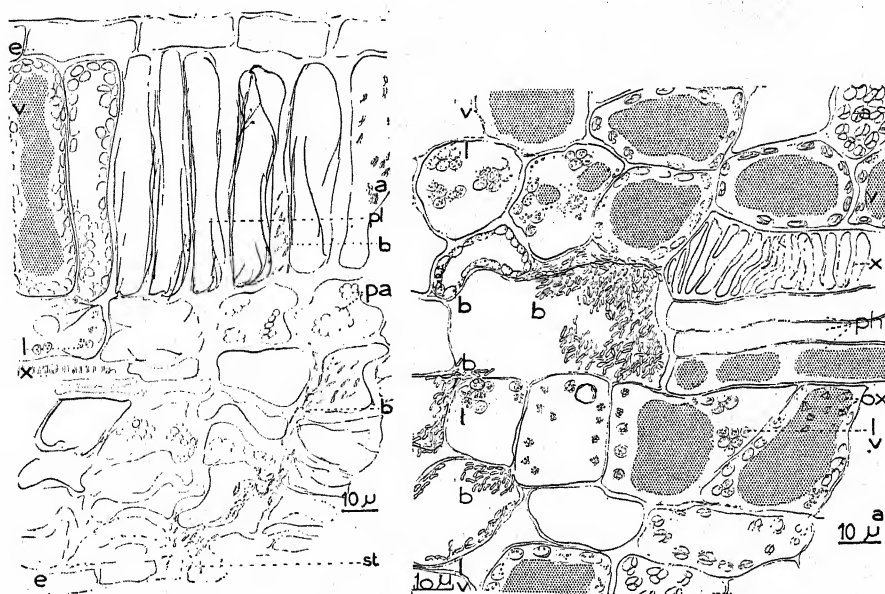


FIG. 2 (Right). Free-hand transverse section through collapsed tissue of tobacco leaf 6 days after having been sprayed and inoculated, according to Clayton's technic. e, epidermis; x, xylem; a, starch grains; l, lipid droplets; pa, agglutinated chloroplasts; pl, plasmolyzed cells; v, vacuolar solution, staining red with neutral red in living cell (shaded in the drawing); b, bacteria; st, stomata.

FIG. 3 (Left). Free-hand transverse section through perivascular tissues of tobacco leaf. x, xylem; ph, phloem; a, starch grains in amyloplasts; v, vacuolar solution (shaded area in the drawing); ox, calcium oxalate crystals floating in the vacuolar solution; l, lipid droplets in chloroplasts; b, bacteria.

The pathological modifications in cell metabolism, and consequently in cell structure of *Bacterium tabacum* in the leaf, depend, as has been observed by Dufrénoy,<sup>2</sup> on the relative humidity of the atmosphere and the relative intensity of sunlight. When the sky was overcast and the relative humidity

<sup>2</sup> Dufrénoy, J. Modifications pathologiques du métabolisme cellulaire chez les tabacs. Ann. Epiphyties. 18: 258-318. 1932.

remained between 100 per cent and 80 per cent, the affected cells remained turgescient, although they became deficient in starch and sugars: as the starch was translocated out of the plastids, oil droplets developed that adsorbed the oil-soluble yellow carotene pigments (Fig. 1, l). "The disintegration of the chlorophyll made the yellow carotene pigment visible, and hence a yellow halo spot was formed"<sup>3</sup>; proteic granules (Fig. 1, pr) also appeared within the disintegrating plastids.

When the sky was clear and the relative humidity was lower than 80 per cent, the vacuolar solution became rich in phenolic compounds (Fig. 1, t) and in tetrahedric crystals of calcium oxalate, while the pH of the solution rose above 5.

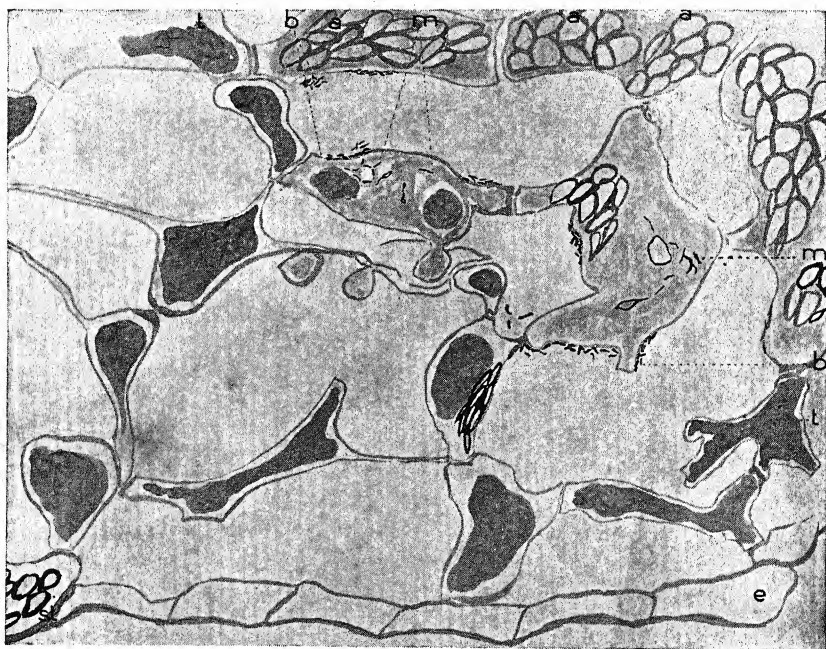


FIG. 4. Transverse section of spongy parenchyma from sprayed and inoculated leaf of tobacco, killed in the Némec fluid, stained by acid fuchsin and polychrome blue. e, epidermis; st, stomatal cell (filled with starch); m, mitochondria; a, amyloplast; t, tannic material in vacuoles; b, bacteria.

A few hours of bright insolation may cause affected cells to dry up and shrink while they still are densely packed with starch-containing plastids; these clump together around the flocculated vacuolar material, wherein the phenolic compounds oxidize into dark brown material.

Cells that survive, on the border of the lesion, revert to a meristematic

<sup>3</sup> See footnote 1.

<sup>4</sup> See footnote 2.



condition; plastids thin out into rod-shape mitochondria as the starch grains are hydrolyzed into glucids, which are used up for respiration.

Enhanced respiratory activity is made possible by exaggeration of contact surfaces in the affected cells, as cytoplasm spreads into films around a number of small vacuoles; a concomitant shifting of the vacuolar pH from 4.5 towards 6, suggests that glucids (part of which were incompletely oxidized into organic acids to be stored in the vacuolar sap of the normal cells) are now, as has been noted by Dufrénoy,<sup>5</sup> more completely oxidized to carbon dioxide.



FIG. 5. Transverse section of tobacco leaf at the margin of *Bact. tabacum* lesion. e, epidermis; pl, plasmolyzed palisade cells, each containing flocculated tannic vacuolar material; ox, calcium oxalate crystals; v, vacuolar solution staining red in the vacuole of living cell (shaded area in figure); pv, vacuolar precipitate, floating in the vacuolar solution; n, nucleus staining with neutral red in dying cell; p, nucleus showing as a bright unstained sphere in living cell; p, plastids; pa, agglutinated plastids; l, lipid droplets in plastids; b, bacteria.

#### HALO LESIONS

The necrotic spot marking the locus of either needle-prick or natural inoculation by *Bacterium tabacum* is surrounded by a "halo" when bacteria remain alive in an area, but are unable to rapidly invade and destroy the adjacent cells, thus giving the toxin time to diffuse ahead.<sup>6</sup> This occurs during periods of high humidity or light rains (Fig. 1).

<sup>5</sup> See footnote 2.

<sup>6</sup> Layton, E. E. Water soaking of leaves in relation to development of the wildfire disease of tobacco. Jour. Agr. Research [U. S.] 52: 239-269. 1936.

## EPIDEMIC WILDFIRE

The lesions of epidemic wildfire differ from those of halo wildfire in that, due to water-soaking, tissue invasion proceeds at such a rate that the adjoining tissues are killed.

Cells reverting to the meristematic condition can still be detected at the margin of epidemic wildfire, lesions, as in the case of halo wildfire. These meristematic cells may be most susceptible to bacterial invasion.

Tobacco leaves submitted to a strong water spray for 2 minutes develop water-soaked areas that recover without injury, if not subsequently inoculated, but that may develop into "experimental wild fire lesions," if a suspension of *Bacterium tabacum* be atomized (Figs. 2-3).

Infected water-soaked areas show no conspicuous changes as long as cloudy humid weather prevails, although, within 24 hours after inoculation, bacteria can be seen swarming in intercellular spaces in sections of water-soaked tissues (Fig. 4). Thereafter, a few hours of strong insolation may cause the water-soaked, infected area to dry up and darken into typical wildfire lesions (Fig. 5).

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## PHYTOPATHOLOGICAL NOTES

*Chemical Treatments Helpful in Germination Tests of Seeds.*<sup>1</sup>—During the past 5 years several hundred samples of cereal grains, variously treated as well as nontreated, have been submitted to the Division of Seed Investigation at Geneva, New York, for germination and disease studies. Usually those treated with Ceresan, New Improved Ceresan, and Sanoseed have developed higher percentages of normal sprouts than have the nontreated, formaldehyde-treated or copper-treated samples. Even more noteworthy, however, the fungous growths common and confusing on seed germination trays, *Alternaria* sp., *Fusarium* spp., *Helminthosporium* spp., and *Rhizopus nigricans* have been consistently absent from the germinated seeds treated with one of the mercury compounds. Growths of *Rhizopus nigricans* have completely obscured the developing seedlings of nontreated rye. When an organic mercurial has been applied, easily readable tests (Fig. 1) have been secured. In supplementary experiments, zinc oxide, formaldehyde dusts, and several copper compounds have not inhibited the development of these organisms appreciably unless applied at dosages that induced seedling abnormalities.

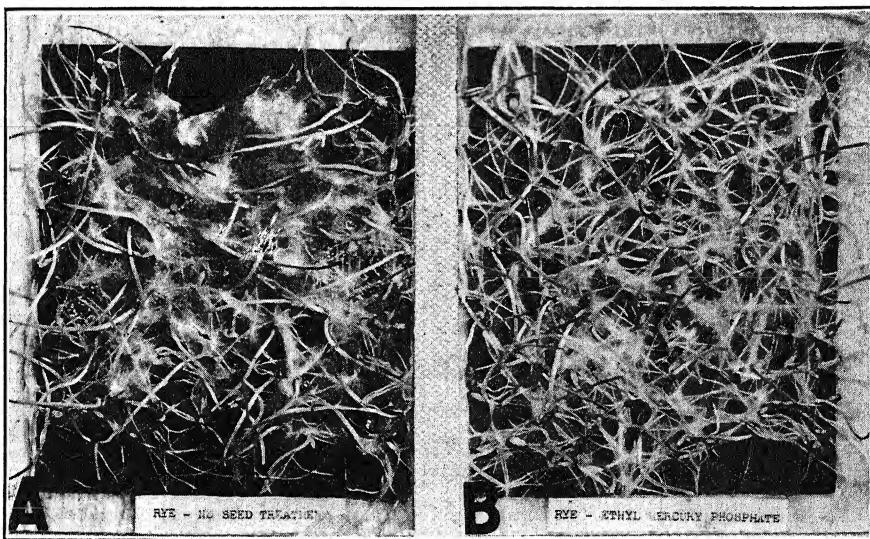


FIG. 1. Seeds of rye germinating on wood-pulp blotters at 20° C. A. Vigorous growths of *Rhizopus nigricans* developing from nontreated seed. B. Fungus development prevented by application of New Improved Ceresan.

<sup>1</sup> Approved by the Director of New York State Agricultural Experiment Station as Journal Paper No. 184.

Similarly, many germination samples of peas, beans, and corn appear to need the protection afforded by one of the organic mercury compounds. Immature, decadent, and dead seeds of these crop plants are very favorable media for soft-rot bacteria and for several fungi of the profuse-growth type. In our experiments, treatments of the samples with the mercury compounds have prevented the development of such associated microorganisms, while not affecting the seedlings as to percentages of germination, green weight, color, length of radicles or of plumules, or development of secondary roots. This means that the treatments have increased decidedly both the speed and accuracy of appraising the germination tests. With the possible exceptions of copper oxalate and a dilution of cuprous oxide, materials other than organic mercurials have not been considered satisfactory for this purpose.

Our laboratory now regularly uses as a standard treatment, when germinating certain small grains, beans, corn, peas, and other large seeds, a very reduced strength of ethyl mercuric phosphate, obtained by mixing 1 part of N. I. Ceresan with 5 parts of French talc. This dust protectant has been found to be applied easily to any dry seed and to adhere well during subsequent handling of the seed. The usual procedure is to shake the seeds and chemical together in a stoppered flask or a small screw-top bottle. The excess dust must be removed completely; a small screen or test-tube basket is useful for this purpose. The seeds can be held in storage for several days or placed to germinate immediately without fear of mercury-induced aberrations. Other proprietary mercury compounds, Ceresan, Merko, Sanoseed and Semesan, have been used successfully at their full strength.

The mercury materials can be applied as dips also, and usually with a saving of time as compared to the dust applications. Efficiency seems not to be sacrificed. An 0.18 per cent aqueous mixture of N. I. Ceresan has proved to be a dependable therapeutic. The dry seeds can be placed in the liquid, shaken to remove air bubbles, immediately poured over a tea strainer, and placed on the germination towels. Simply pouring the mixture over the seeds in the tea strainer ordinarily insures a sufficient adherence of mercury. A 1.1 per cent mixture of Ceresan, of Sanoseed, or of Semesan, or a 3 per cent mixture of Merko is, in some cases, a workable substitute, but these materials are more difficult to keep in suspension and are considerably more expensive.—WILLARD CROSLER, STEWART PATRICK, AND LLOYD TAYLOR, New York State Agricultural Experiment Station, Geneva, N. Y.

*A Further Note on the Fungus Causing a White Foot Rot of Wheat and Oats.*—The eyespot or elliptical type of lesion at the bases of culms of wheat and oats in the coastal counties of Oregon is caused by a fungus that produces a dirty-white, loose mycelial growth on potato-dextrose agar. After the initial growth, the cultures develop stromatic pads or sclerotia, which resemble

those produced by races of *Rhizoctonia solani* Kühn. Since the symptoms of the disease were not typical of those expected for *Rhizoctonia* and did resemble those described for *Gibellina cerealis* Pass., an attempt was made to obtain pure cultures of the latter fungus. Cultures alleged to be *G. cerealis* were obtained from Italy, at that time the only source for them, and, since they proved to be identical with the Oregon fungus, the latter was tentatively assigned to this species.<sup>1</sup> During the following years, attempts to produce or find a fruiting stage of the fungus were unsuccessful. In 1934, there was no appreciable freezing weather, hence the disease appeared earlier than usual.<sup>2</sup> While it was observed that the fungus attacked the leaves of cereals and velvet grass, *Holcus lanatus*, in a manner similar to the way in which *Rhizoctonia solani* attacks turf grasses in producing brown patch,<sup>3</sup> yet the use of the name *Gibellina cerealis* was tentatively continued<sup>2</sup> for the reasons stated above. In later unpublished reports and in the check list of graminicolous diseases in Oregon,<sup>4</sup> the unsettled status of the name of the fungus continued. Subsequent work has now shown the fungus does not belong in the genus *Gibellina*.

The first authentic culture of *Gibellina cerealis* Pass. was obtained by Glynne<sup>5</sup> from wheat growing in plots at the Rothamsted Experiment Station, Harpenden, England, in 1936. The fungus produced a mounded, slow-growing, eventually pale grey colony, in which numerous perithecia developed. This was the final proof necessary to show that the tentative assignment of the Oregon fungus to *Gibellina* was incorrect; and that both the fungus from Oregon and the one from Italy possibly were referable to the genus *Rhizoctonia*. The writer wrote to Miss Glynne, who very kindly sent him specimens of *Gibellina cerealis* on wheat collected at the Rothamsted Experiment Station. She had previously obtained cultures of the fungus from Oregon, which the writer had isolated and tentatively named *Gibellina cerealis* Pass. for reasons stated above, and had found this identification erroneous. Indications at present are that the fungus is a species of *Rhizoctonia*.—RODERICK SPRAGUE, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, cooperating with the Oregon State Agricultural Experiment Station, Corvallis, Oregon. Pub-

<sup>1</sup> Sprague, R. Preliminary note on another foot rot on wheat and oats in Oregon. *Phytopath.* 24: 946-948. 1934.

<sup>2</sup> —. Observations on diseases of Gramineae in Oregon and adjacent parts of Washington during the open winter of 1933-1934. U. S. Dept. Agr. Bur. Plant Indus. Plant Disease Rptr. 18: 24-25. 1934. [Mimeographed.]

<sup>3</sup> Monteith, J., Jr., and A. S. Dahl. Turf diseases and their control. *Bull. Green Sect. U. S. Golf Assoc.* 12: 87-186. 1932.

<sup>4</sup> Sprague, R. A preliminary check list of the parasitic fungi on cereals and other grasses in Oregon. U. S. Dept. Agr. Bur. Plant Indus. Plant Disease Rptr. 19: 156-186. 1935. [Mimeographed.]

<sup>5</sup> Glynne, Mary D. Some new British records of fungi on wheat: *Cercospora herpotrichoides* Fron., *Gibellina cerealis* Pass., and *Ophiobolus herpotrichus* (Fr.) Sacc. *Brit. Mycol. Soc. Trans.* 20: 120-122. 1936.

lished as Technical Paper No. 263 of the Oregon Agricultural Experiment Station.

*Seed Transmission of Tomato Mosaic Following the Planting of Freshly Extracted Seed.*—In October, 1934, an unusual amount of mosaic appeared in young tomato plants in breeding experiments conducted by W. S. Porte, in a greenhouse at the Arlington Experiment Farm. The plants were grown from seed planted immediately after extraction from fruits harvested in the field. Soon after transplanting, 13 of the 257 plants showed mosaic. Precautions had been taken to prevent infection from without, and the circumstances suggested seed transmission, since mosaic occurred on many plants in the field whence the seed was obtained. To test the possibility of seed transmission, tomatoes were collected from greenhouse plants showing ordinary mosaic (tobacco virus 1) and from those showing the streak caused by tobacco mosaic combined with the potato X-virus. One portion of the seed (A) was washed and dried for 8 days, and the other portion (B) was extracted from the fruit without washing and planted immediately. Both lots were planted November 16, and the seedlings transplanted singly in 4-inch pots on November 29. Pots and soil had been steam-sterilized and the plants were grown in benches, surrounded by a barrier of woven wire to prevent contact. Plants from seed from mosaic-free tomatoes were used as controls. No other plants were grown in the house, and it was fumigated each week.

On December 11, 6 of 249 plants in Series A, from seed of mosaic plants, were stunted and bore twisted, filiform cotyledons. Four plants out of 136, also in Series A, but from seed of streaked plants, were similarly affected. In Series B, identical symptoms occurred in 8 of 257 plants from seed of mosaic plants, and in 7 of 104 plants from seed of streaked plants. On January 5, all plants showing this cotyledon deformity had developed 4 to 5 leaves and showed typical mosaic symptoms. No streak appeared at any time and no additional cases of mosaic occurred after December 11. The 523 controls remained healthy. These results seem to offer definite evidence of seed transmission of mosaic in seed planted soon after extraction from the fruit.

Seeds from plants affected either with mosaic or with streak were stored from 3 to 12 months before planting, and plants grown from this seed were handled in the greenhouse as described above. These tests included 3,567 plants, none of which showed any evidence of mosaic or streak during the seedling stage. Nineteen of these plants, however, did show symptoms of mosaic after they had developed 4 to 6 leaves. None of the plants showed symptoms of streak at any time, and the 937 control plants remained healthy. These results differ in two important respects from those obtained with fresh

seed, since (1) there was a much smaller percentage of infection in the plants grown from stored seed, and (2) the symptoms in the infected plants did not appear until they were long past the seedling stage. The factors responsible for the late appearance of mosaic in these plants have not yet been satisfactorily determined and further work is in progress. The writers feel, however, that the infection in the plants grown from stored seed cannot at present be definitely attributed to seed transmission of the virus.

The present note seeks to emphasize the possibility of seed transmission of mosaic in breeding work, where, to produce several generations of plants in close succession, seed is planted soon after its extraction from the fruit. Where this is done there seems to be a definite danger of an appreciable amount of seed transmission of the virus.—S. P. DOOLITTLE and F. S. BEECHER, U. S. Horticultural Field Station, Bureau of Plant Industry, Beltsville, Md.

*False "Black Chaff" of Wheat Produced by Inoculating with Stem Rust.*—The so-called "black-chaff" disease of Hope and H-44 wheats, which appears to have been confused often with the true black-chaff disease caused by *Bacterium translucens* var. *undulosum*, or attributed to such organisms as *Phytomonas atrofaciens*, *Colletotrichum graminicolum*, and *Alternaria*, has recently been demonstrated to be, at least in part, the result of a peculiar reaction to infection by *Puccinia graminis tritici*. This reaction appears to be confined to varieties or hybrid strains of wheat having a specific type of mature plant resistance to this disease. Microscopic examinations of the dark-color lesions suggest that the discoloration may be the result of disintegration of the tissues of the invading rust organism within the living cells of the host.

Hypodermic inoculations with stem rust of  $F_2$  plants of an H-44  $\times$  Marquis cross show that all plants that give the "black chaff" reaction are resistant to stem rust in the mature-plant stage. Both macroscopic and microscopic examinations showed that all plants were infected. Since this includes both homozygous and heretozygous resistant plants, the "black chaff" reaction can be used in some crosses as an "ear mark" for identifying, previous to the blooming stage, plants that carry the factor for one type of mature-plant resistance to stem rust. This may simplify breeding for rust resistance, especially by the back-cross method.—E. S. McFADDEN, Associate Agronomist, United States Department of Agriculture.



# STUDIES OF THE PATHOGENICITY OF *PHYSALOSPORA OBTUSA*<sup>1</sup>

H. H. FOSTER<sup>2</sup>

(Accepted for publication April 20, 1937)

## INTRODUCTION

It is generally accepted that *Physalospora obtusa* (Schw.) Cooke (*Sphaeropsis malorum* Pk.), the causal organism of black rot of apple (24) induces a serious spotting of the leaves, as well as fruit rot and canker. However, while certain investigators have been able to obtain infection of apple leaves by inoculation with spores of this fungus, others have reported only negative results. In view of this lack of agreement in the results of earlier investigators and the paucity of knowledge regarding the details of infection and the conditions that favor or limit it, it seemed desirable to undertake the studies herein reported, using potted plants and equipment that permitted partial control of certain environmental factors.

Scott and Rorer (22) proved conclusively through inoculation experiments that *Sphaeropsis malorum* induced leaf spot of apple under orchard conditions in southern Missouri. From the results of leaf-inoculation experiments, Brooks and DeMeritt (2) concluded that they were dealing with several "strains" of *S. malorum* varying in general vigor and in their ability to induce leaf spot. They found that a large-spore form with single-locule, ostiolate pycnidia induced the highest percentage of leaf spot. In Maine, C. E. Lewis (12) concluded that *S. malorum* was able to induce leaf infection in the orchard when the leaves were inoculated early in the season and under favorable conditions. Crabill (7), working in Virginia and using several fungi, obtained leaf infection in the greenhouse with *S. malorum*, and concluded that both the initial infection and enlargements of frog-eye leaf spots were caused by this fungus. I. M. Lewis (13), however, working in New Hampshire, failed to obtain infection from orchard inoculations. Hesler (10), in New York, conducted leaf-inoculation experiments extending over a period of 4 years, but obtained only negative results, except in a few cases where wounds and moisture were provided. His results indicated no correlation between morphological or biological characters of the fungus and pathogenicity. Hesler (10) also suggested that I. M. Lewis (13) and C. E.

<sup>1</sup> This paper is an abridgment of a thesis presented to the Graduate School of the University of Wisconsin in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

The writer is grateful to Dr. G. W. Keitt, under whose direction these investigations were performed, for advice and criticism during the work and preparation of the manuscript. Acknowledgment is made to Eugene Herrling for photographing the illustrative material.

<sup>2</sup> Junior Plant Pathologist, Division of Tobacco and Plant Nutrition, Arlington Experimental Farm (Va.).

Lewis (12), in explaining their results, failed to consider the question of physiologic races. Walton (18, 27) reported only negative results from inoculations made in Pennsylvania under a variety of conditions and over a period of years.

#### LEAF INOCULATION STUDIES

Inoculations with pycnospora suspensions were made on 25 commercial apple varieties to determine the ability of certain isolates of *Physalospora obtusa* to induce leaf infection.

TABLE 1.—*Isolates of Physalospora obtusa used for leaf-infection studies*

Isolate No.	Source	Furnished by	Date of isolation
1	Culture—apple wood—Virginia	Berg, A.	8/9/31
2	Culture—diseased apple leaf—Virginia	Berg, A.	6/14/31
3	Culture—ascospores from apple—Virginia	Shear, C. L.	
4	Culture—ascospores from <i>Prosopis</i> —Honolulu, Hawaii	Shear, C. L.	1927
4-a	Single spore derived from culture 4		
8	Apple fruit—variety unknown—Maine	Folsom, D.	12/11/31
8-a	Single spore derived from culture 8		10/5/32
9	Culture— <i>Cydonia sinensis</i> —France	Arnaud, G.	10/8/32
9-a	Single spore derived from culture 9		1/7/34
9-b	Single spore derived from culture 9		
12	Apple leaf—variety unknown—S. Carolina	Armstrong, G. M.	5/12/32
12-a	Single spore derived from culture 12		10/8/32
15	Culture—Kalbas pear fruit—S. Africa	Dippenaar, B. J.	5/19/30
15-a	Single spore derived from culture 15		10/5/32
18	Fameuse apple fruit—Sturgeon Bay, Wis.	Blodgett, E. C.	9/27/32
18-a	Single spore derived from culture 18		10/8/32
19	Apple fruit—variety unknown—Univ. Orch.		10/9/32
19-a	Single spore derived from culture 19		12/3/32
20	Dudley apple fruit—Sturgeon Bay, Wis.	Blodgett, E. C.	10/10/32
21	Apple fruit—variety unknown, Madison, Wis.	Honey, E. E.	11/1/32
22	Haralson apple fruit, Whalan, Minn.	Langord, L.	10/24/33
23-2	Golden Delicious apple fruit—Maine	Folsom, D.	11/4/33
23-7	" " " " "	"	"
24-1	Rome Beauty apple fruit—Winchester, Va.	Cooley, J. C.	10/28/33
24-2	" " " " "	"	"
25	Stayman Winesap apple fruit " "	"	"
30	Ben Davis apple fruit " "	"	11/2/33
32	Tolman apple fruit—Sturgeon Bay, Wis.	Blodgett, E. C.	11/2/33
33	Wolf River apple fruit—Sturgeon Bay, Wis.		10/29/33
34	Wealthy apple fruit " " "	"	"
35-2	Duchess apple fruit " " "	"	"
37-2	Dudley apple fruit " " "	"	10/30/33
38	Wealthy apple fruit " " "	"	"
39-2	Northwestern Greening apple fruit " "	"	"
40	Fameuse apple fruit—Sturgeon Bay, Wis.	"	"
43	McMahon apple fruit " " "	"	"
45	Culture—Jonathan apple fruit—Mich.	Cation, D.	
47-q-2	Quince fruit—Amherst, Mass.	Davis, W. H.	1/26/34
47-q-3	Quince fruit " "	"	"
48	Baldwin apple fruit—Amherst, Mass.	"	"
50	Apple canker—variety unknown—Mich.	Cation, D	4/1/34



### Materials and Methods

*Spore Production and Inoculation.* Pycnospores used for leaf inoculations were produced on potato-dextrose agar cultures in 6- and 12-ounce bottles. Cultures used for inoculation, listed in table 1, were kept in alternating diffused light and darkness, either in the laboratory in front of a window or in the greenhouse, certain experiments having indicated that such lighting favored sporulation. Sufficient pycnidial production usually occurred within 8 to 16 days. In figure 1 are shown 6 14- to 16-day-old cultures that had developed pycnidia. The spore suspension was obtained by removing the fungal mat from the agar, placing it between 2 or 3 layers of cheesecloth, and crushing out the spores in distilled water. Spore production varied among the different isolates, the spore suspension from some cultures containing only an occasional spore per low-power field of the microscope. An average of 10 to 15 spores per low-power field of the microscope was the most concentrated suspension used. The inoculum was applied to the apple leaves by means of a clean De Vilbiss atomizer operated by compressed air. Both the dorsal and ventral leaf surfaces were inoculated during the first greenhouse season, but atomizing the upper leaf surface was discontinued when it was determined that penetration occurred through the stomata.

Preliminary leaf inoculations were made in the spring of 1932. After inoculation, trees were kept in a moist chamber, devised by Keitt (11), at graduated temperatures from 20° to 28° C. for 24- to 48-hour periods, in order to determine a favorable temperature and period in the moist chamber for leaf infection. Trees thus kept for 48 hours at temperatures of 24° to 28° C. often showed a leaf spotting apparently caused by some physiological disturbance. During the latter part of the first greenhouse season and thereafter a 24-hour period in the moist chamber at 20° C. was adopted as the standard treatment following inoculation (8). Trees kept as controls showed no infection and no harmful effects from this treatment in the moist chamber, while abundant leaf spotting developed on inoculated trees. The causal organism frequently was reisolated at will.

*Care of the Trees.* In most of these studies 2-year-old potted nursery apple trees were used. Two or 3 shoots per tree were allowed to develop. The trees were, as far as feasible, kept in the same greenhouse and under similar environmental conditions. Trees having a similar amount of shoot growth were used for a given series. After the trees were removed from the moist chamber they were left on the greenhouse bench at least 14 days before final data were taken.

*Method of Taking Data.* The total number of lesions was recorded per  $\frac{1}{2}$  sq. in. of maximally infected area per leaf. In case the lesions had coalesced to the extent of covering an area of  $\frac{1}{2}$  sq. in., the number of lesions was estimated as 100; if the coalesced area covered was  $\frac{1}{4}$  sq. in., the number of

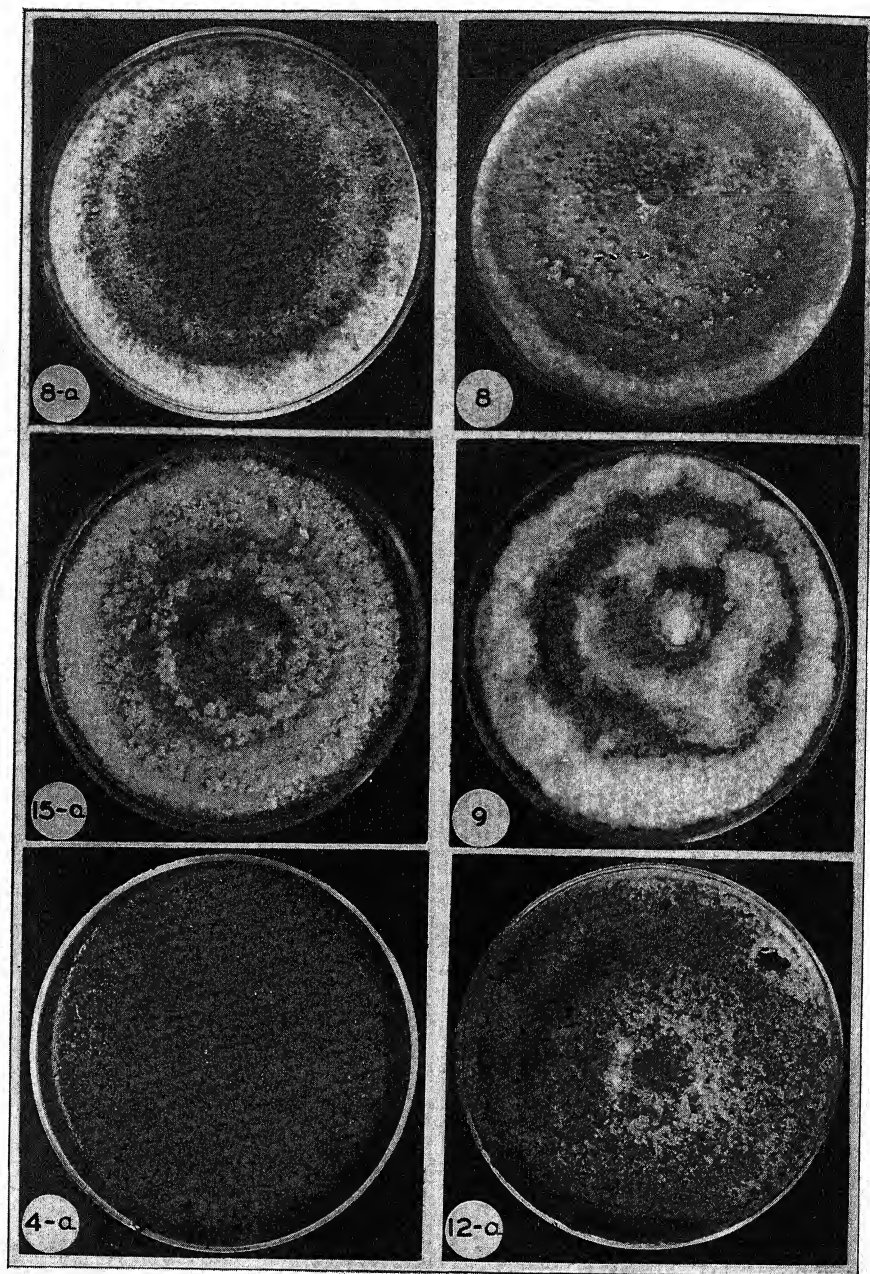


FIG. 1. Six 14- to 16-day-old isolates of *Physalospora obtusa* grown on potato-dextrose agar at room temperature.

TABLE 2.—*Comparison of pathogenicity of 7 isolates of Physalospora obtusa on certain apple varieties*

Variety	Average number of lesions per maximally infected 1/2 square inch per leaf for stated isolates and numbers of tests <sup>a</sup>													
	Isolate 8-a		Isolate 8		Isolate 9		Isolate 12-a		Isolate 15-a		Isolate 18-a		Isolate 19-a	
	Tests	Isolate	Tests	Isolate	Tests	Isolate	Tests	Isolate	Tests	Isolate	Tests	Isolate	Tests	Isolate
	No.		No.		No.		No.		No.		No.		No.	
Baldwin .....	22	44	1	...	1	0	...	...	...	...	...	...	1	1
Rome Beauty .....	28	61	3	1	3	0	1	...	0	...	...	...	3	2
Fameuse .....	31	56	4	...	2	0	...	...	...	...	...	...	2	2
Wealthy .....	35	48	3	2	3	0	1	...	0	...	0	...	4	0
Mammoth Black Twig .....	37	42	2	...	3	1	...	...	...	1	0	...	3	0
McIntosh .....	45	41	4	...	2	0	...	...	...	...	...	...	2	0
Ben Davis .....	46	48	3	2	9	0	1	...	0	1	0	...	3	1
Northwestern Greening .....	58	70	3	2	4	1	1	1	0	1	...	...	3	1
Yellow Transparent .....	59	51	3	2	5	0	1	1	0	1	0	...	3	0

<sup>a</sup> Each test represents data on 1 tree per respective culture with the exception of Baldwin, in which case 2 trees were used for each 8-a, 8, and 19-a.

lesions estimated was 50. When leaves showed less than 50 lesions per  $\frac{1}{2}$  sq. in., actual counts were made. It is clearly recognized that these results are to be regarded as semiquantitative.

### Comparison of Seven Isolates on Nine Apple Varieties

*Experiments of 1933.* Seven cultures, 5 of which were derived from single spores, were used in pathogenicity trials (Table 2). The 2 Maine isolates, 8 and 8-a, induced comparatively severe infection. The French isolate 9 induced comparatively light infection on the 5 apple varieties inoculated. The South Carolina isolate 12-a induced a slight amount of infection on 3 of the 7 varieties. Of all the cultures used 12-a proved to be the most variable in pathogenicity. The Wisconsin isolate 19-a induced a slight amount of infection on 5 of the 9 varieties. Isolate 15-a from South Africa and isolate 18-a from Wisconsin failed to induce infection on the varieties inoculated. Reisolation of isolates 8, 8-a, 9, 12-a, and 19-a were made from one or more of the apple varieties during the 1933 greenhouse season. From the data shown in table 2 it is evident that isolates vary in respect to pathogenicity, some inducing consistent and abundant infection, while others result in little or no infection.

### Comparison of 27 Isolates on Northwestern Greening and Yellow Transparent

Thirty additional isolations were made in the fall and winter months of 1933 from diseased material received from different parts of the United States. In an attempt to obtain more information regarding the occurrence and distribution of leaf-infecting isolates, 27 cultures were used in leaf

TABLE 3.—*Comparative pathogenicity of certain leaf-infecting isolates of Physalospora obtusa*

Isolate	Northwestern Greening			Yellow Transparent		
	Tests	Trees	Av. no. lesions per maximally infected $\frac{1}{2}$ sq. in. per leaf	Tests	Trees	Av. no. lesions per maximally infected $\frac{1}{2}$ sq. in. per leaf
	<i>Number</i>	<i>Number</i>		<i>Number</i>	<i>Number</i>	
12-a .....	4	7	3	2	4	3
23-2 .....	3	3	45	2	2	54
23-7 .....	2	3	18	1	1	1
24-1 .....	1	1	7	1	1	26
24-2 .....	1	1	12	1	1	25
25 .....	3	3	67	1	1	51
30 .....	2	2	73	2	2	81
47-q-2 ....	1	1	64	.....	.....	.....
47-q-3 .....	1	1	20	1	1	33
48 .....	2	2	17	1	1	2

inoculations on Northwestern Greening and Yellow Transparent varieties during the 1934 greenhouse season. Only 10 isolates induced definite leaf infection (Table 3). From culture 12-a only a sparse infection resulted on both Yellow Transparent and Northwestern Greening varieties. Nine of the isolates listed in table 3 were obtained from material received from Maine, Massachusetts, and Virginia; these induced varying amounts of infection. Twelve isolates used in this experiment were obtained from Wisconsin, 2 from Michigan, and 1 from Minnesota. The failure to obtain more than occasional sparse infection from isolates obtained from Wisconsin, Michigan, and Minnesota indicates to the writer that leaf-infecting isolates occur less frequently in this area than in certain of the Eastern States. This indication is supported by many references in the Plant Disease Bulletin to the occurrence of frog-eye leaf spot in the Eastern States and few references to its occurrence in the upper Mississippi Valley.

Regarding the variation in ability of isolates to induce leaf infection, Orton and Wood<sup>3</sup> state: "The writer (Orton) and his associates have for several years failed to secure infection in Pennsylvania with isolations of *Sphaeropsis* from various sources. Whether there is a difference between the frog-eye diseases as they appear in different parts of the country remains to be shown." Butler (4) reported the isolation of "strains" of *Sphaeropsis malorum* differing in pathogenicity. Hesler (9) and Shear (23) also suggested the possibility of variation among isolates. It seems possible that the apparently frequent occurrence of non-leaf-infecting isolates in nature, at least in certain sections of the country, may explain in part the negative results obtained in certain experiments by I. M. Lewis (13), C. E. Lewis (12), Brooks and DeMerritt (2), Hesler (10), Orton and Wood<sup>3</sup> and Walton (18, 27).

#### Relation of Temperature in the Moist Chamber to Infection

An experiment was undertaken to determine the amount of infection induced by isolate 8-a on Yellow Transparent and Northwestern Greening trees kept in the moist chamber at graduated constant temperature ranging, at 4-degree intervals, from 4° to 32° C. Trees of each variety were inoculated in duplicate and placed in the moist chamber for a 24-hour period at each of the 8 temperatures. The average amount of infection induced at the respective temperatures is shown in figure 2.

Macroscopic symptoms, under the conditions of this experiment, appeared on leaves kept in the moist chamber at temperatures from 8° to 28° C., indicating a comparatively wide temperature range in which infection may occur.

<sup>3</sup> Orton, C. R., and Jessie I. Wood. "Frog eye," black rot, and New York apple tree canker. U. S. Dept. Agr. Bur. Plant Indus. Plant Disease Repr. Sup. 33: 61-63. [Mimeographed.] 1924.

A high percentage of spores of isolate 8-a germinated between 12° and 32° C., as shown in figure 3, A. However, the optimal temperature for the elongation of germ tubes of isolate 8-a, under the conditions of this experiment, was 24° C., with a comparatively high average extending from 20° to 32° C. (Fig. 3, B).

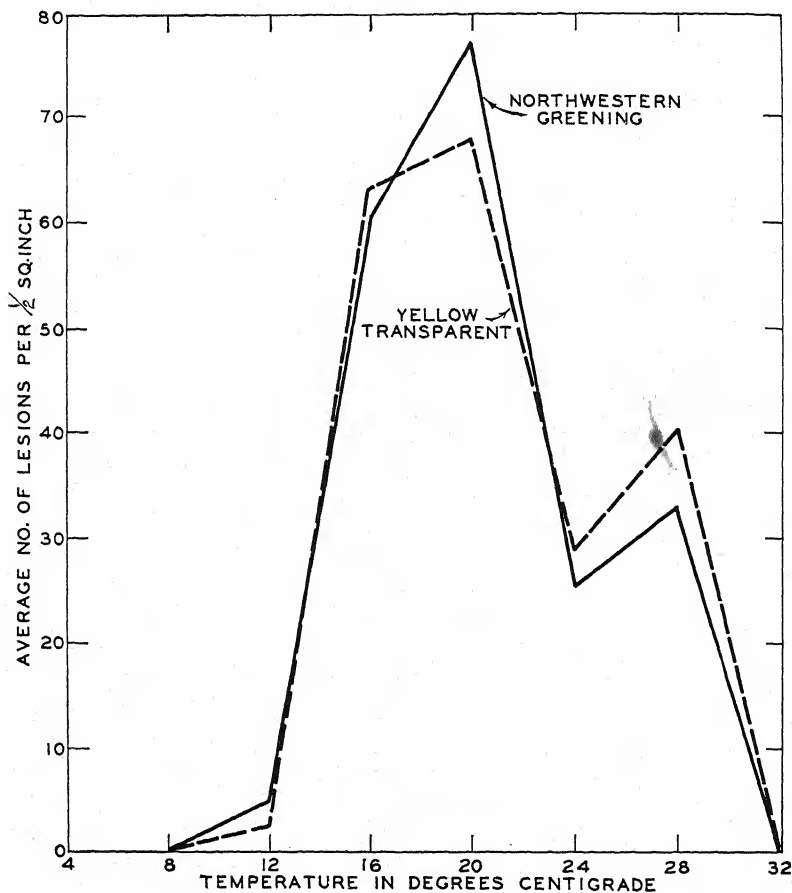


FIG. 2. The relation of temperature in the moist chamber to amount of leaf infection induced by isolate 8-a on Yellow Transparent and Northwestern Greening.

When inoculated Yellow Transparent and Northwestern Greening trees were kept in the moist chamber for 48 hours at 8° C., considerable leaf infection developed. On 2 Yellow Transparent trees an average of 9 lesions developed per  $\frac{1}{2}$  sq. in. per leaf, while 2 Northwestern Greenings showed an average of 40 lesions. Spore germination may occur at 8° C. (Fig. 3, A), although more time is required for the germ tubes to elongate and for penetration to take place at this low temperature.

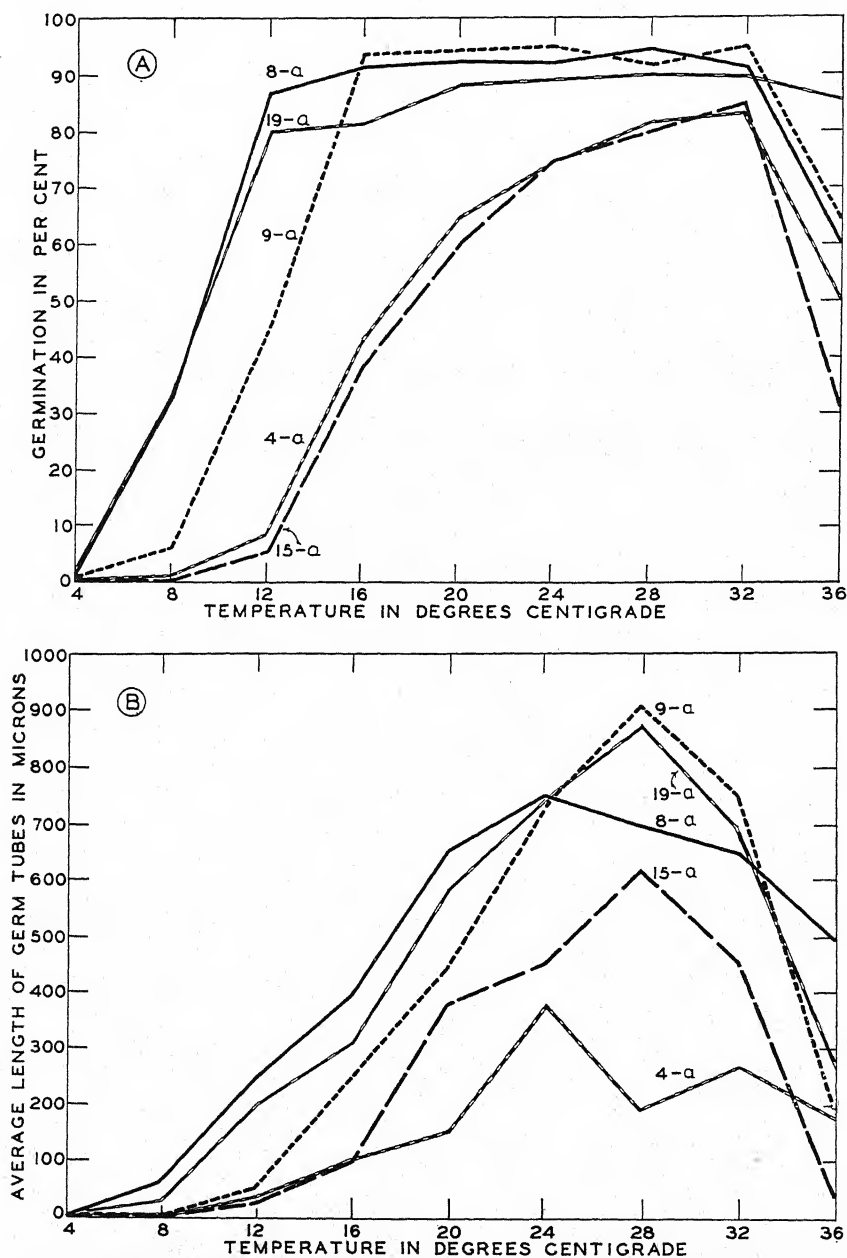


FIG. 3. Effect of temperature on 5 cultures. A. Percentage germination. B. Average length of germ tubes in microns.

## Relation of Period in the Moist Chamber to Infection

Following inoculation, trees were kept in the moist chamber at 20° C. Two trees were removed at each 4-hour interval of 24 consecutive hours. The amount of infection induced by isolate 8-a on Yellow Transparent during the 1934 greenhouse season is shown in figure 4. In addition an experiment to determine the amount of infection induced by isolate 8-a on Northwestern Greening was made during the 1933 greenhouse season and repeated during that of 1934. An average of these 2 series also is shown in figure 4.

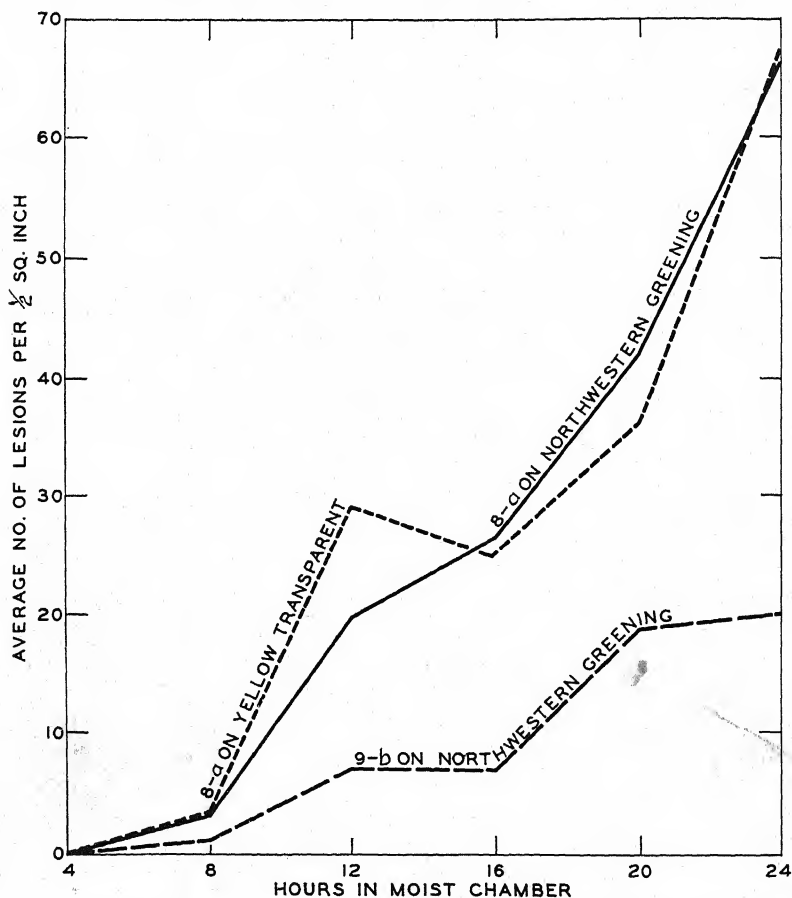


FIG. 4. Relation of period in moist chamber to amount of leaf infection. The results from 1 test are shown for 9-b on Northwestern Greening and for 8-a on Yellow Transparent. The results from 2 series are shown for 8-a on Northwestern Greening.

A minimal period of 8 hours in the moist chamber at 20° C. was necessary for the initiation of infection. The graph representing the infection induced by isolate 8-a on Northwestern Greening shows a rather gradual but con-



sistent increase in the amount of infection developing from 8 to 24 hours in the moist chamber at 20° C. Infection induced by isolate 8-a on Yellow Transparent follows a similar trend, except that the infection following the 12-hour period in the moist chamber was somewhat greater than that following the 16-hour period, a result thought to be aberrant. The infection induced by isolate 9-b on Northwestern Greening was much less than that induced by isolate 8-a on the same variety. However, the same general trend is shown, the greatest amount of infection developing after 24 hours in the moist chamber at 20° C. Trees inoculated with isolate 8-a and placed in the moist chamber at 20° C. for more than 24 hours often showed a high percentage of the leaves dead when the trees were removed from the chamber. Since a gradual development of the disease was considered desirable, 24 hours in the moist chamber at 20° C. was set as the maximum moist period at this temperature in these studies. The data of figure 4 suggest that in nature considerable infection may be initiated within 12 to 16 hours under conditions of favorable moisture and temperature.

#### APPEARANCE OF MACROSCOPIC SYMPTOMS

*Incubation Period.* Since the leaf spot induced by *Physalospora obtusa* has been described by a number of investigators, a detailed description of symptoms will not be given. However, the time after inoculation when macroscopic symptoms first appear has received but little attention. Among the investigators who obtained definite leaf infection, Crabill (7) reported that leaf spotting was first noticed, under greenhouse conditions, 16 days after inoculation and that 15 days later enlargement of the spots began. C. E. Lewis (12) reported some infection on apple seedlings and young Baldwin trees, under greenhouse conditions, but no mention was made of the time when macroscopic symptoms first appeared. He inoculated McIntosh leaves in the orchard on June 2 and 3, but did not observe spots until July 1. Scott and Rorer (22) stated that inoculations were made in the orchard on May 28, and that on June 3, 6 days later, purple specks were appearing. By June 18 the leaves were badly spotted.

In the writer's greenhouse studies several isolates induced consistent leaf infection. These isolates fall into at least 2 groups as to the time of the first appearance of macroscopic symptoms. Under optimal conditions 8-a, 24-1, 24-2, 25, and 30 always induced some macroscopic lesions within 24 hours after inoculation. Within this group, symptoms induced by isolates 8-a, 25, and 30 were more evident at first appearance. In figure 5, G and H, is shown typical infection induced by isolate 8-a on Yellow Transparent 36 hours after inoculation. Infection on the older, more mature leaf shown in figure 5, G, was scarcely visible 36 hours after inoculation, while the younger, immature leaf (Fig. 5, H) was heavily infected, the lesions having coalesced to a large

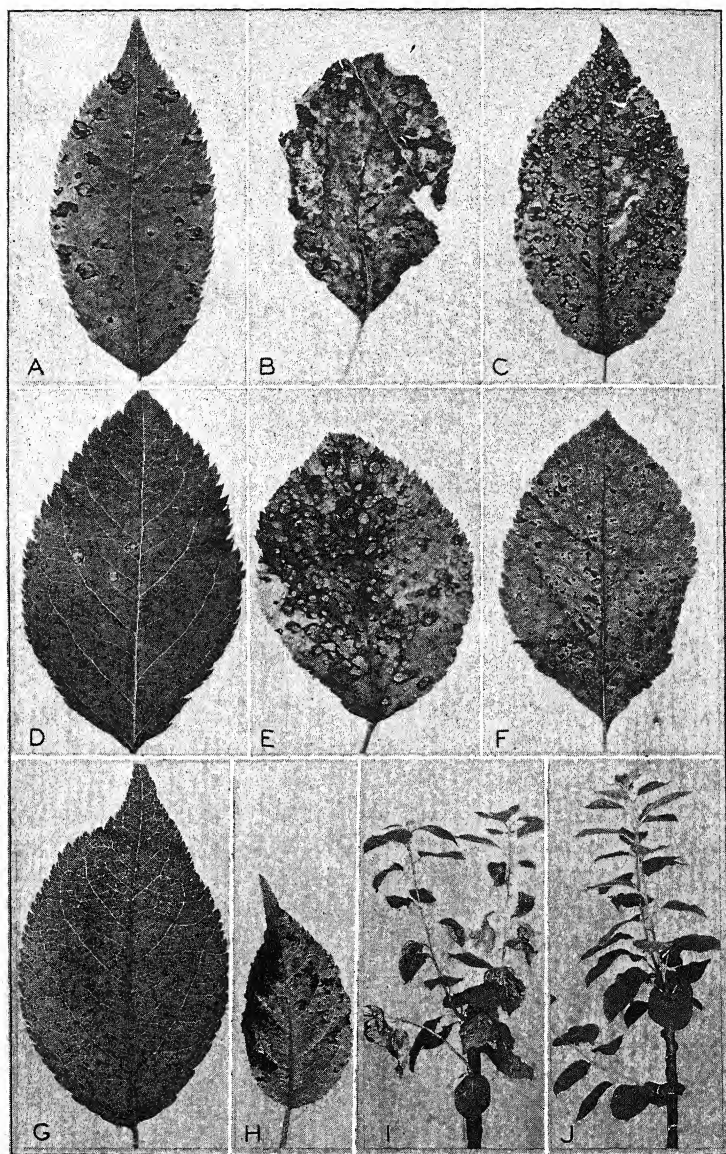


FIG. 5. A-C. Severe infection induced by isolate 8-a on Virginia (crab), Anoka, and Northwestern Greening, respectively. D-F. Infection induced by isolate 9-b on Virginia (crab), Anoka, and Northwestern Greening, respectively. G and H. Typical infection, 36 hours after inoculation, induced by isolate 8-a on a nearly mature leaf and on a younger, more actively growing leaf of Yellow Transparent. I and J. The result of dorsal (I) versus ventral (J) leaf surface inoculation on Yellow Transparent.

extent. Under similar conditions isolates 23-2 and 47-q-2 induced slight macroscopic symptoms about 2 days after inoculation, while macroscopic symptoms induced by isolates 9, 9-b, 23-7, 47-q-3, and 48 were first observed 3 to 4 days after inoculation. Whether or not the symptoms induced within a 24-hour incubation period were the result of a toxic secretion produced by the fungus following stomatal penetration and acting in advance of the fungus hyphae was not determined.

*Infection on Younger vs. Older Leaves.* Frog-eye leaf spot was observed by I. M. Lewis (13), M'Cormack (14, pp. 142-143), and Chase (5, pp. 40-41), to make its first appearance, or to be more severe on the younger, more actively growing apple leaves. Walton (26) reports, as a result of extensive bagging experiments conducted over a period of 2 years, that leaf infection may take place during the blooming period, but most of it occurs from the time the petals fall until  $2\frac{1}{2}$  weeks later, or when the leaves are in their most actively growing condition. Also, meteorological records showed that frog-eye infection usually was correlated with periods of rainfall when the temperature was sufficiently high for spore germination. Scott and Rorer (22), Brooks and DeMeritt (2) and Quaintance and Scott (19, pp. 35-37) observed that, although leaf infection first appeared early in the spring, the leaves apparently remained susceptible throughout the growing season. In artificial inoculations on York Imperial leaves, Crabill (7) found that the old leaves developed 5 times as many spots as the young leaves. The writer found leaf spot to be more abundant on the younger, more actively growing leaves than on the older leaves of the shoot. Coalescing of spots, resulting in more severe infection, also was of more general occurrence on the younger leaves (Fig. 5, H).

*Pycnidial Production.* Scott and Rorer (22), M'Cormack (14, pp. 142-143), and Quaintance and Scott (19, pp. 35-37) have reported sparse pycnidial production on infected apple leaves attached to the tree. Zeller (28), writing of leaf spot in Oregon, stated that pycnidia seldom matured on the leaves. Pycnidial fructification on fallen leaves has been reported by Scott and Rorer (22), M'Cormack (14, pp. 142-143), Quaintance and Scott (19, pp. 35-37), and Bryce (3).

During the course of these greenhouse studies the writer failed to observe mature pycnidia on infected leaves while they were attached to the shoot or after they had fallen. In many cases pycnidial formation appeared to have been initiated but when these dark, slightly raised bodies were crushed and examined under the microscope, no pycnosporos were observed. Infected leaves bearing these apparently immature pycnidia in the center of the original lesion were placed over moistened filter paper within Petri dishes. After the leaves remained in these improvised moist chambers, exposed to alternating diffused light and darkness for a period of 1 to 2 weeks, many

of the pycnidia were found to contain mature pycnospores. This suggests that humidity may play an important rôle in pycnidial development.

### Comparative Susceptibility of Apple Varieties

Varietal reaction to leaf spot has been noted by several investigators. Crabill (7) stated that no variety of apple observed was immune from frog-eye but that some were more susceptible than others. Reed and others (20) reported that Ben Davis and Black Twig were among the varieties more severely attacked, while York Imperial and Winesap were less susceptible. Reed and Crabill (21) reported Ben Davis, Black Twig, Winesap, and "Albemarle Pippin" as the most susceptible varieties. Crabill (7) states: "Of the very susceptible varieties, Ben Davis, Winesap, Arkansas, Baldwin and Jonathan are noteworthy. Early Harvest, Grimes, Yellow Newtown (Albemarle Pippin), Gano and York Imperial are among the least susceptible." Orton and Wood<sup>4</sup> listed certain susceptible varieties as follows: Stayman, Transparent, and Strawberry from Delaware; Ben Davis and Delicious from Kentucky; Maiden Blush from Illinois; Jonathan from Kansas, and *Malus ioensis* from New York. Britton and others (1) observed that Baldwin was the most susceptible variety, although others showed some infection. Walton (26) noted but little difference in the susceptibility of varieties. Definite leaf infection from pycnospore inoculations was reported by C. E. Lewis (12) to occur on Baldwin and McIntosh foliage.

During the 1934 and 1935 greenhouse seasons 22 varieties of apple trees were tested, in 3 trials, for comparative susceptibility of leaves to *Physalospora obtusa*. Two isolates, 8-a and 9-b, each inducing leaf infection, but

TABLE 4.—Comparative susceptibility of apple varieties to 2 monosporic cultures of *Physalospora obtusa*

Variety	Av. no. lesions per maximally infected $\frac{1}{2}$ sq. in.		Variety	Av. no. lesions per maximally infected $\frac{1}{2}$ sq. in.	
	No. 9-b	No. 8-a		No. 9-b	No. 8-a
Virginia (crab).....	17 (1) <sup>a</sup>	46 (6)	Haralson .....	45 (12)	83 (20)
Rome Beauty .....	18 (2)	11 (1)	Grimes .....	47 (13)	23 (3)
Mammoth Black			Winesap .....	51 (14)	76 (18)
Twig .....	22 (3)	14 (2)	Anoka .....	58 (15)	74 (16)
Jonathan .....	25 (4)	51 (7)	Northwestern Green- ing .....	59 (16)	87 (21)
Whitney (crab).....	33 (5)	39 (5)	Red June .....	61 (17)	.....
Duchess .....	36 (6)	69 (13)	Wealthy .....	.....	70 (14)
Early Harvest .....	36 (7)	61 (9)	Turley .....	.....	76 (19)
Gano .....	39 (8)	75 (17)	Willow Twig .....	.....	71 (15)
Delicious .....	42 (9)	61 (10)	Yellow Transparent	.....	69 (12)
McIntosh .....	42 (10)	52 (8)	Liveland Raspberry..	.....	38 (4)
Patten Greening ....	45 (11)	63 (11)			

<sup>a</sup> Numbers in parentheses indicate rank in relative susceptibility.

<sup>4</sup> See footnote 3.

differing from the other in certain respects, were used in these trials. Under the conditions of this experiment no variety was found immune. The results of the first test are shown in table 4. The amount of infection apparent on several of the varieties varied considerably in these 3 tests. Certain varieties, however, appeared to be noticeably less susceptible than others. This was particularly noticeable with the inoculation results for isolate 9-b. In 2 out of 3 trials Virginia (crab) proved to be the least susceptible, and in the third test was among the less susceptible varieties. Anoka was very susceptible in all 3 tests, while Northwestern Greening was very susceptible in the first and only moderately so in the remaining 2 tests. In figure 5, A to F, are illustrations of infected apple leaves. The infection was induced by isolate 8-a and 9-b, during the first test on Virginia, Anoka, and Northwestern Greening. The leaves showing infection induced by isolate 9-b were photographed 26 days after inoculation, while those showing infection induced by isolate 8-a were photographed 36 days after inoculation. It is clearly shown that Virginia is markedly less susceptible than Northwestern Greening or Anoka. Although isolate 8-a may induce considerable leaf spot on Virginia, the resulting infection is noticeably less severe than on Northwestern Greening and Anoka. Virginia appeared definitely less susceptible to both isolates 8-a and 9-b. The leaf spots were fewer in number and many of the individual spots appeared as flecks or aberrant lesions surrounded by a light-color halo with a diffuse margin. Yellowing of the foliage and premature defoliation were induced on Anoka by both isolates.

The writer is inclined to agree with Crabill (7) that probably no apple variety is immune from a consistent leaf-spotting "strain" of the fungus. In these greenhouse studies rather marked variation in susceptibility was observed in the reaction of different varieties; also, a noticeable difference in the varietal reaction induced by the 2 isolates. It seems probable that, in nature, variation in the extent to which varieties are attacked may be explained in part by the occurrence of different "strains" or physiologic races of the fungus, together with climatic variations and differences in the age and condition of the foliage.

#### Mode of Penetration

*Cleared-Leaf Method.* A modification of the Peace (16) method was used for a study *in toto* of spore germination and penetration because it offered a means of observing germinating spores and germ tubes that had penetrated stomata upon a relatively large area of the leaf surface. Yellow Transparent, Northwestern Greening, and certain other varieties were inoculated and placed in the moist chamber for 24 hours at 20° C. Isolate 8-a was used in these studies, which extended over the 1933 and 1934 greenhouse seasons. Portions of leaves were removed 24 and 48 hours following inocu-

lations. Small pieces were placed in a preparation of equal parts of glacial acetic acid and 100 per cent alcohol until the chlorophyll was removed. The leaf material was cleared in a saturated chloral hydrate solution and stained with acid fuchsin in lacto-phenol.

Leaf material from Yellow Transparent, Northwestern Greening, and to a limited extent certain other varieties, was examined. Both young, actively growing leaves and older, mature leaves were examined for stomatal penetration. The older leaves proved to be superior for this study, primarily because of their less excessive pubescence, which enabled one to follow the course of the germ tube much more readily. In all cases observed penetration was found to take place through the stomatal opening. Germ tubes often extended for a distance of several hundred microns before entering a stomatal opening. In no cases were appressoria observed. Figure 6

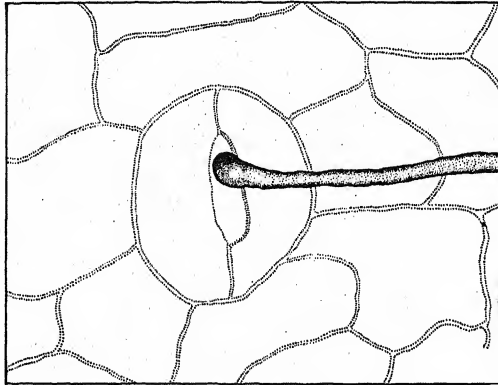


FIG. 6. Camera-lucida sketch of stomatal penetration by a germ tube of *Physalospora obtusa*, from a cleared Yellow Transparent leaf, 48 hours after inoculation, examined *in toto*.  $\times 64$ .

shows a camera-lucida drawing illustrating stomatal penetration. No definite evidence of infection was apparent in preliminary experiments where the leaf tissue was wounded prior to inoculation.

*Dorsal vs. Ventral Leaf Surface Inoculation.* During the spring of 1933 an inoculation experiment was made to aid in determining the mode of penetration. Two Yellow Transparent and two Wealthy trees were used in this experiment. A pycnosporous suspension of isolate 8-a was atomized on the dorsal leaf surface of 1 tree of each variety, while the other 2 trees were inoculated on the ventral surface. The results of this experiment showed that an average of 36 lesions per  $\frac{1}{2}$  sq. in. per leaf developed on the Wealthy that had been inoculated on the dorsal leaf surface, while no infection developed on the tree inoculated on the ventral surface. On the Yellow Transparent tree inoculated on the dorsal leaf surface an average of 77 lesions per  $\frac{1}{2}$  sq. in. per leaf were counted, while on the tree inoculated on the ventral surface



an average of three lesions per  $\frac{1}{2}$  sq. in. per leaf were observed (Fig. 5, I, J). It seems possible that the small amount of infection appearing on the Yellow Transparent tree inoculated on the ventral leaf surface resulted through an accidental transfer of the inoculum to the dorsal surface, though pains were taken to avoid this. The writer considers these results as strong confirmatory evidence that penetration of the fungus occurs chiefly, if not entirely, through the stomata.

#### APPLE FRUIT INFECTION STUDIES

Limited studies of fruit infection were made, with the primary aim of comparing pathogenicity on leaves with that on fruits. The black rot of apple fruits was reported first by Peck (17, pp. 20-21) in 1879. A review of the literature reveals a rather extensive list of reports and descriptions of the disease in the various apple-growing districts of the world.

Variation among isolates of the black-rot fungus in their ability to infect apple fruits has been reported by a number of investigators. Walker (25) reported a form of *Sphaeropsis malorum* that produced pycnidia lacking ostioles. She stated that this form induced rot more rapidly on fruits than did the recognized ostiolate forms. Cultural differences also were noted. Brooks and DeMeritt (2) found that "strains" varied in their ability to infect apple fruit. The "first strain," which induced a higher percentage of leaf infection than other "strains," rotted green fruit quite as readily as ripe fruit. The "second strain" was unable to infect green apples and produced rot more slowly on mature fruit. The "third strain" also produced rot more slowly than the "first strain." By inoculating 9 different varieties of apple fruits with the same culture Hesler (10) showed variation to occur with reference to decay, pycnidial production, and the formation of concentric rings. He also found that different "strains" varied in their ability to infect the same variety. Cooley and Fenner (6) carried on investigations more than 2 years, using several varieties of apples. In all, 155 cultures from 14 different localities were tested. They found as much variation, as to the size of the rot produced, within a group from a certain locality as between the groups from different localities. Mohendra and Mitra (15), reporting on variation in the cultural behavior of *Sphaeropsis malorum*, found that both types of cultures under investigation appeared to be equally active in parasitism. They, however, noted that the number of pycnidia per unit area of surface of the parasitized fruit was about 3 times as great in the one case as in the other. With the exception of Mohendra and Mitra (15), the above-mentioned investigators have found more or less variation among isolates in their ability to induce rot on apple fruit. Zeller (28), however, found no indication of variation among his isolates. He reported that the same type of rot of apple fruits was obtained with fungus cultures isolated from leaf spot, bark canker, and fruit rot.

## Materials and Methods

The pathogenicity of all leaf-infecting isolates as well as certain monosporic cultures not inducing leaf infection was tested upon one or more varieties of apple fruits.

*Production of Inoculum.* From 3- to 5-day-old mycelial growth was used as inoculum. The cultures were grown on potato-dextrose agar and the mycelial growth was obtained just back of the growing margin.

*Treatment of Apples.* The apples were washed in soap suds and water, rinsed in 95 per cent alcohol, immersed in a 10 per cent solution of commercial B-K for a few minutes, then rinsed in sterile distilled water and allowed to dry. In the process of inoculation, a disc 7 mm. in diameter and approximately 10 mm. in depth was removed from each apple midway between the blossom and stem ends by means of a cork borer. Three 6-mm. discs of mycelium were inserted into each apple and the disc of fruit was replaced. The apples were kept in the laboratory for 14 days. During the several tests the temperature ranged from 17° to 24° C. The average temperature was approximately 20° to 22° C. Seven days after inoculation the external

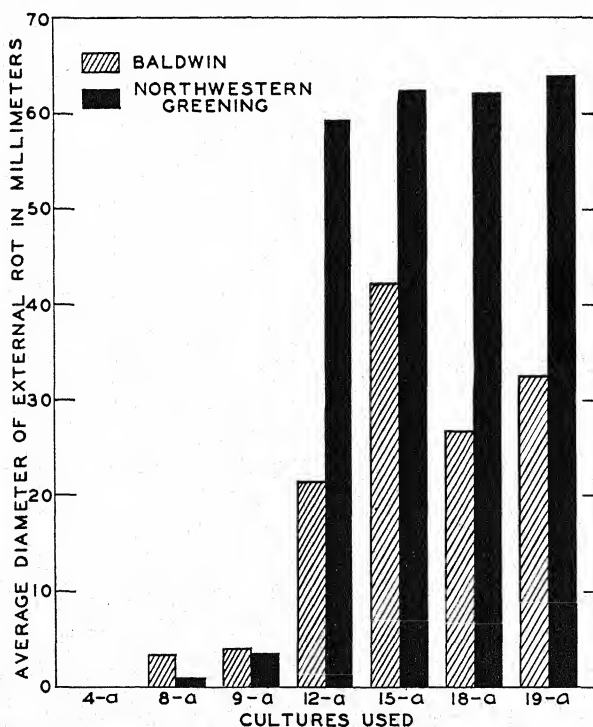


FIG. 7. Average, from 2 series, of external rot induced by certain monosporic cultures of *Physalospora obtusa* on Baldwin and Northwestern Greening apple fruits. The data are based upon a total of 10 to 12 apples per culture, with the exception of 19-a, in which case only 6 Baldwin apples were used in 1 test.



diameter of the rotted area was measured and additional notes were taken after a period of 14 days.

Jonathan, Baldwin, Northwestern Greening, and Winesap varieties were used in a limited number of fruit-infection tests. All isolates that induced leaf infection, and in addition certain monosporic isolates, were tested on Winesap. All leaf-infecting isolates, with the exception of 8, 8-a, and 9-b, induced external rot on Winesap fruit varying between 3 and 6 cm. in diameter. A comparison of the external rot induced by certain monosporic isolates on Baldwin and Northwestern Greening apple fruits is shown in figure 7. Isolate 4-a failed to show any evidence of inducing rot. Isolates 12-a, 15-a, 18-a, and 19-a induced a greater amount of external rot on Northwestern Greening. Isolates 8-a and 9-a induced rot slowly on both varieties, though the diameter of the external rot was somewhat greater on the Baldwin apples.

Leaf-spotting isolates 8, 8-a, and 9-b induced but little rot on apple fruits. Other leaf-spotting isolates, however, induced rapid rotting of apple fruits. Brooks and DeMeritt (2) reported that the "strain" that induced the highest percentage of leaf infection also induced the most rapid fruit rot. The fruit-infection studies conducted by the writer, though somewhat limited, show that some of the most virulent leaf-spotting isolates induced the least amount of fruit rot in a given period. Isolate 8, from a rotted apple fruit obtained from Maine, induced frog-eye leaf spot within 24 hours after inoculation, but induced rot on apple fruit very slowly. Isolate 23-7, also obtained from a rotted fruit from Maine, induced rather consistent leaf infection and a rapid rot on apple fruits. Isolate 12, isolated from a diseased apple leaf from South Carolina, always induced fruit rot but was never consistent in the amount of leaf infection induced. From these results it would seem that there is no definite correlation between the ability of isolates to induce leaf spotting and black rot of fruit.

#### SUMMARY

Preliminary experiments showed that a 24-hour period in the moist chamber at 20° C. offered approximately optimal conditions for initiation of apple-leaf infection by *Physalospora obtusa*.

Seven isolates, 5 of them monosporic, were used in leaf-infection studies on 9 apple varieties. Isolates 8, 8-a, and 9 induced infection on all varieties inoculated. Isolates 12-a and 19-a induced a slight amount of infection on certain varieties, while isolates 15-a and 18-a failed to induce macroscopic symptoms on any variety inoculated.

Twenty-seven isolates from different regions of the United States were used in leaf inoculations on Northwestern Greening and Yellow Transparent. Only 10 isolates induced definite leaf infection. The results of these experi-

ments indicate that leaf-infecting isolates occur less frequently in the upper Mississippi Valley than in certain of the Eastern States.

The relation of temperature in the moist chamber to leaf infection induced by isolate 8-a Northwestern Greening and Yellow Transparent was determined. Macroscopic symptoms were apparent on trees kept at temperatures from 12° to 28° C., with maximal infection developing on trees kept at 20° C.

The amount of leaf infection developed in relation to the period in the moist chamber was determined for isolates 8-a and 9-b on Northwestern Greening and for isolate 8-a on Yellow Transparent. An 8-hour period in the moist chamber, following inoculation, was the minimal period necessary for the development of macroscopic symptoms. The maximal amount of infection under the conditions of this experiment, occurred following a 20-hour period in the moist chamber.

The minimal incubation period of the isolates that consistently induced leaf infection varied from 20 to 96 hours.

Lesions were found to be more numerous and coalescing of spots to be of more general occurrence on the young, actively growing leaves than on the more mature leaves.

When leaves bearing immature pycnidia were placed over moistened filter paper within Petri dishes, mature pycnosporos usually developed within 1 to 2 weeks.

Twenty-two varieties of apple trees were tested for comparative susceptibility to 2 isolates. Although none proved immune, certain varieties were consistently less susceptible than others.

Stomatal penetration by the fungus was determined by using a modification of the cleared-leaf method of Peace and by dorsal *versus* ventral leaf surface inoculations.

From the results of inoculations with several varieties of apple fruits it was apparent that no definite correlation existed between the ability of isolates to induce frog-eye leaf spot and black rot of fruit.

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# THE FEEDING OF THE ROOT-KNOT NEMATODE IN ROOT TISSUE AND NUTRIENT SOLUTION<sup>1, 2</sup>

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## INTRODUCTION

The manner in which the root-knot nematode, *Heterodera marioni* (Cornu, 1879), Goodey, 1932, obtains its food has remained obscure up to the present, despite the fact that this economically important and biologically interesting parasite has been studied intensively from many points of view. Christie (1) recently has considered the relationship of parasite to host and the development of nematode galls, with an extensive review of prior work. Briefly, the larva penetrates a root at or near the tip, migrates inward and comes to rest with its head in an intercellular space, usually in the plerome. Here it remains throughout life, in the case of a female. Host cells in the vicinity of the head enlarge, becoming densely granular and multinucleate, and it is assumed that the parasite obtains its food chiefly or entirely from these, the so-called giant cells. Both the manner of feeding and the nature of stimuli leading to development of giant cells and galls have been subjects of speculation based chiefly upon observation of sections of fixed material.

## LITERATURE REVIEW

To account for pathological states induced by the feeding of several plant-infesting nematodes, various workers have postulated an outpouring of some secretion from the mouth. Goodey (3) in a recent review of diseases caused by nematodes, credits Ritzema Bos with first postulating such a secretion hypothesis to explain pathogenesis in the case of *Ditylenchus dipsaci* (Kühn, 1857), Filipjev, 1936. Among more recent workers Kostoff and Kendall (4), in particular, expanded this hypothesis to account for the formation of giant cells around the head of *Heterodera marioni*. This general hypothesis was accepted by Christie (1) in his recent studies as the most tenable one. Such secretion is assumed to pour from the mouth into intercellular spaces since, as Christie states, "The head of the parasite lies between the cells and there is no evidence that the stylet pierces adjacent walls." This secretion is assumed, among other actions, to increase permeability of the cells so that the parasite may obtain its food by sucking up fluid from the intercellular spaces.

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<sup>2</sup> The cost of publication of this paper was borne by the Pineapple Producers Cooperative Association, Ltd.

The first actual observation of outpouring of a secretion from a salivary gland (the dorsal esophageal gland) through the stylet of a nematode was reported recently by Linford and Oliveira (6) for two predacious species of *Aphelenchoides*. Linford (5) showed this particular secretion to initiate the process of digestion within the prey prior to ingestion by the predator.

Protrusion of the stylet of *Heterodera marioni* seems not to have been recorded for live specimens, and sections of fixed root tissue have yielded no evidence that the stylet punctures cells. As recently as 1935, in fact, Goodey (3) stated for stylet-bearing nematodes in general: "... there seem to be no records of direct observations of the stylet actually functioning as a puncturing organ" although its structure suggests such a function. More recent work, however, has presented numerous examples of puncturing by stylets in several genera of nematodes.

Christie and Arndt (2) described the penetration of fungal hyphae by the stylets of *Aphelenchus avenae* Bastian, 1865, and *Aphelenchoides parietinus* (Bastian, 1865), Steiner, 1932, but stated that the stylet did not remain appreciably protruded during feeding. Linford (5) later observed the stylet to remain protruded to the maximum extent during the full period of activity of the esophageal bulb in Hawaiian cultures of these species. Linford and Oliveira (6), reporting the predacious habit in species of 3 dorylaim genera and in 2 species of *Aphelenchoides*, found the stylet used effectively in puncturing prey and held far protruded during the entire period of activity of the muscular esophagus. Linford (5) extended this observation to include a third predacious *Aphelenchoides* as well as a fungus-sucking form, *Ditylenchus intermedius* (de Man, 1880), Filipjev, 1936.

The observations on this last-named nematode require detailed review here, for, although free-living, this species approximates the sessile parasites in its feeding. Otherwise an active nematode, it lies motionless for periods of one to two hours after its stylet penetrates the fungal cell before its esophageal bulb begins its rhythmic pulsation; then, after a few seconds, it pulls its stylet out of the hypha and moves away. Injection of saliva into the fungus was not seen, but an anterior drift of saliva along the duct dorsad of the lumen of the esophagus to near the base of the stylet, soon after penetration of the hypha, was observed repeatedly. By analogy with the predacious *Aphelenchoides*, it appears that this nematode begins the digestion of its food within the fungal cell, reducing the protoplasm to a consistency that may be drawn in through the extremely slender lumen of its stylet. By comparison, *Aphelenchus avenae* and *Aphelenchoides parietinus*, which suck out hyphal contents immediately, have coarse stylets.

The apparent discrepancy between current assumptions that *Heterodera marioni* does not penetrate cells with its stylet and the writer's observations on many species of free-living nematodes that not only puncture with their

stylets but hold them protruded during the entire period of ingestion of food, led to an attempt to see how the root-knot nematode feeds. The actual feeding of larvae and adult females in live root tissue has now been witnessed and the activities accompanying feeding have been followed in live females removed from galls. The latter observations are the more complete and are here reported first.

#### METHODS

For the observation of nematodes removed from the root, swollen females after their last molt were teased from simple galls in the lateral roots of pea, *Pisum sativum* L., transferred to a fluid medium, and examined without a cover glass under a water-immersion objective. Several simple media were tried, but a solution of dextrose, 2 per cent, plus peptone, 2 per cent, proved so satisfactory that it has been used extensively and no attempt has been made to improve upon it. Great care is required to disengage the nematode from gall tissues without injury, even in pea roots. Still greater difficulty is presented by roots in which secondary thickening occurs.

Live nematodes in root tissue were observed at a magnification of 600 diameters in relatively thick sections with the water-immersion lens and a powerful but narrow beam of light. Larvae in migration and early stages of swelling were observed in longitudinal sections cut at random through heavily infested root tips of pineapple. Later stages, up to almost the beginning of egg deposition, were obtained from simple galls on lateral roots of pea by means of sections cut with careful orientation. Working in water on transparent celluloid under a wide-field microscope with transmitted light, the positions of nematode body and giant-cell group were judged as closely as possible, and then two cuts were made as close to the nematode as possible without injuring it. For this, a small hollow-ground surgical knife was most satisfactory. To see feeding clearly it is essential that, on one side at least, the giant-cell group be cut through near the head. This was not accomplished with fully mature females without injuring the nematode, but numerous favorable sections were obtained with slightly younger parasites.

All such sections were examined without a cover glass, since, repeatedly, the placing of a cover served to quiet the enclosed nematode even when the cover was supported to withhold pressure from the section.

#### LIVE NEMATODES OBSERVED IN NUTRIENT SOLUTION

Adult female nematodes removed from galls and examined in nutrient solution have shown the following activities: swinging of head, thrusting of stylet, extrusion of saliva, and rhythmic pulsation of the esophageal bulb. Eggs were deposited if this had begun within the root. By changing the mounting medium at intervals to remove accumulated bacteria it was possible

to continue observations over long periods. The nematode shown in figure 1 was held under intermittent observation for 5 full days, and, when discarded, still showed normal activity of head, stylet, and bulb. Several others were observed over 48 hours but most were discarded, though alive, after shorter periods.

*Movement of Head and Stylet.* Immediately after removal from the root most of the nematodes that are not visibly damaged have the stylet fully retracted but a few have it far protruded, so that up to a third of its total length extends beyond the head. The head and stylet may be directed forward, in line with the axis of the "neck" region and esophagus, or may be pointed far to one side or another (Fig. 1). Prolonged observations reveal two chief types of activity, one a thrusting of the stylet, the other a swinging of the head. A much slower bending of the entire narrow anterior part of the body is seen in some specimens.

The stylet is moved persistently, at varying intervals being thrust forward, then retracted to its position of rest. In an active specimen these thrusts may be spaced less than 1 second to several seconds apart, many of them bringing the stylet tip just beyond the lips. Periodically, thrusts may become more frequent and vigorous until the stylet is well protruded (Fig. 1, B, C, E), when motion stops for a few seconds or sometimes much longer. The stylet is then gradually retracted to rest briefly before another series of thrusts begins. Such motion of the stylet may occur in whatever direction the head is pointing, even backward as far as 110 degrees from straight forward.

While the stylet is retracted, the head is moved slowly, but with definite muscular control, not only dorsally and ventrally, but also laterally, to either side. Such motion involves little general bending of the whole neck region, but chiefly a shifting of the position of the head accompanying an expansion of folds of the cuticle on one side and extreme contraction of those on the other (Fig. 1, E, F). These movements occur in no definite sequence. A nematode, when first examined after removal from a root, may have its head in any of these positions, most frequently forward, but some time afterwards it starts to move its head. Thus it is evident that in adult females there is a well-developed muscular control of a type not common among nematodes in general and not suspected in the free larvae of this species. Head movements of nematodes, as a group, involve a general bending of the body behind it and are, to a great extent, confined to one plane, being actuated by the dorsally and ventrally placed muscular fields.

If the stylet is found protruded when first seen, it often remains so for periods up to 30 minutes or more, while the head remains motionless. After it is first retracted, it frequently is thrust repeatedly in the same direction, often being held protruded one to several minutes a number of times, at



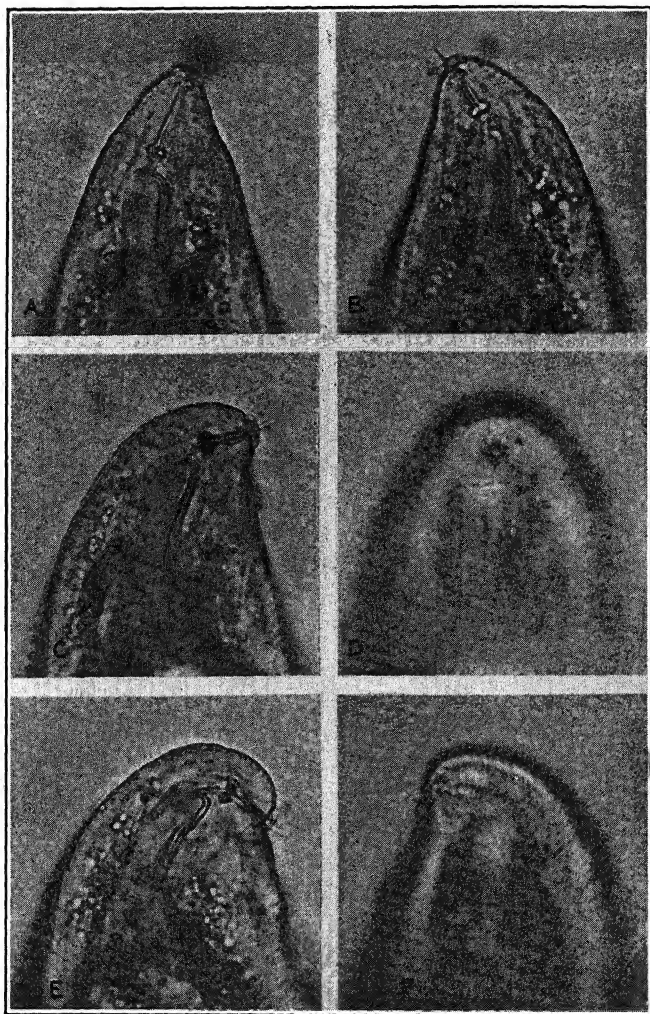


FIG. 1. Mobility of head and stylet of a mature *Heterodera marioni* female, illustrated by photomicrographs of one nematode taken during an hour while the position of the enlarged body (not shown) remained undisturbed. The ventral surface of the parasite lies at the right. A. Head directed forward, stylet retracted. The dark area beyond the lips is a blemish in the photograph. B. Head turned slightly to dorsal side, stylet protruded. C. Head swung to ventral side, stylet protruded. D. Head directed upward, to the nematode's right side, and stylet retracted. Note the six lips, of which the right and left lateral lips are broader than the dorsal and ventral pairs. E. Head swung far ventrally backward, stylet protruded. F. Head swung dorsally and somewhat to the nematode's right, stylet retracted. Note the expansion of folds of cuticle on one side of the head and contraction on the other. All  $\times 750$ .



intervals, before swinging of the head begins. As long as an hour has elapsed with the head in one position, followed later by extreme head mobility and stylet protrusion in all directions.

*Extrusion from the Stylet.* Flow of secretions from the stylet tip has been observed repeatedly but, thus far, only from nematodes taken from the root apparently uninjured and with the stylet protruded when first examined (Fig. 2). It has escaped from the distal end of the stylet only, generally spinning out as an irregular and often coiled thread and adhering to the

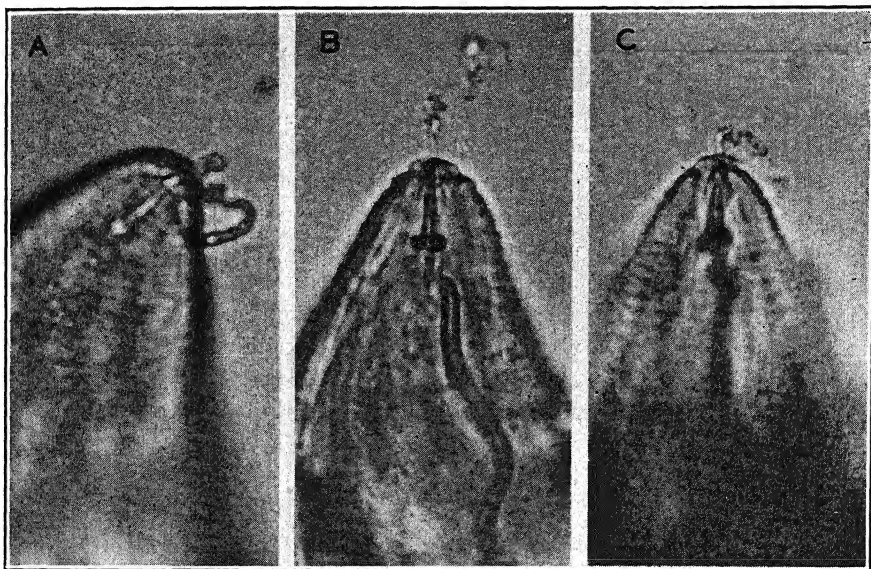


FIG. 2. Anterior ends of 3 *Heterodera marioni* females showing freshly ejected saliva still attached to tips of protruded stylets. A. Nematode in water, with saliva forming a definite but irregular filament. B and C. Nematodes in dextrose-peptone, with saliva forming irregular chains of globules of varied sizes. All  $\times 1250$ .

stylet until that is retracted or the nematode is moved relative to the mounting medium. Such flow has generally commenced promptly, suggesting that it represents a continuation of a process underway at the time of removal from the root. In one instance, after extrusion of a small amount, the stylet was retracted, then protruded for a more copious flow, retracted again, and protruded for a third flow. Four other nematodes exhibited two successive extrusions interrupted by retraction of the stylet. In some others, there were successive flows without retraction of the stylet, lasting in the aggregate for an hour. The maximum flow yet seen equalled little over twice the amount shown in figure 2, B.

That such extrusion from the stylet is not a result of injury is shown by the fact that one of the best flows seen was from a nematode that was still

moving both head and stylet 42 hours after this observation, while others have been from nematodes which, although not observed so long, exhibited normal activity over a long period of hours. One specimen, which was followed 72 hours, extruded secretions at once and then several times later up to 25 hours after removal from the root.

Several individuals that have exhibited no typical extrusion of this nature have shown the slow escape of a small quantity of homogeneous-appearing matter which accumulated as a drop at the stylet tip. This has been seen chiefly in water rather than dextrose-peptone. This may possibly represent food left in the lumen of stylet and of esophagus anterior to the bulb. During escape of the typical secretion, however, granular contents of the dorsal duct have been seen flowing towards the inlet to the lumen of the esophagus and, in a few instances of particularly copious flows, the bulk of the duct content has been visibly reduced. Such observations leave little doubt that the extruded substance corresponds with the secretions postulated by earlier workers and with the digestive secretion injected by predacious Aphelenchoides into their prey. Its designation as saliva seems appropriate.

After escape from the stylet, this saliva appears much more viscous than the content of the dorsal duct. Content of the duct is distinctly fluid, containing minute granules, which sometimes are seen to stream about freely. When saliva pours into water, it usually forms a compact, refractive, coiled filament of irregular diameter and shape (Fig. 2, A), but in a solution of dextrose and peptone, it commonly collects as a series of small droplets that group themselves into a broader and less regular strand streaming away from the stylet tip (Fig. 2, B and C). Coagulation upon contact with plain water is indicated, and it is likely that other media than those tested might produce still other effects.

*Pulsation of the Esophageal Bulb.* In several nematodes which extruded saliva as described and in a few others, the esophageal bulb has been seen to pulsate vigorously in the manner of sucking food. This has been seen only in media containing sugar and much the best in the solution of dextrose and peptone. Such pulsation may start before retraction of the stylet at the conclusion of saliva flow but, after it starts, resumption of saliva flow has been seen in only one specimen. In two nematodes in a solution of dextrose and peptone, there were counted 11 and 8, respectively, separate periods of pulsation ranging in duration from  $3\frac{1}{2}$  minutes down to a few seconds, followed by a long period of quiet. In the nematode shown in figure 1, the bulb was active repeatedly, but at irregular intervals over at least 120 hours after removal from the root. Some nematodes have shown continuous activity for 15 minutes or more. Invariably, however, these periods of pulsation correspond with separate protrusions of the stylet and are separated by at least brief periods during which the stylet is retracted. In every instance, pulsa-

tion began after the stylet was protruded and stopped not later than retraction of the stylet.

#### LIVE NEMATODES IN ROOT TISSUE

A larva of the root-knot nematode in water or agar moves its body actively without thrusting its stylet and with no appreciable motion of its head except that associated with locomotion, but within the root new activity begins. The initial stages of penetration into a root have not been seen, but a nematode with its head between cells of the third layer from the surface, and with the greater part of its body exposed, was seen to thrust its stylet persistently and to swing its head laterally as it struggled to advance. Such head motion was through a much narrower angle than that observed in adult females. Numerous larvae have been seen in stages intermediate between this and the fixed position within theplerome, and all agree in these activities. Most of the stylet thrusts failed to protrude the tip visibly beyond the lips, but in a few instances the tip moved far forward. It was impossible, because of optical difficulties afforded by young root tissue, to determine whether it entered a cell or simply passed between contiguous walls, but no pulsation of the esophageal bulb was detected. It seems probable that migrating larvae use the stylet chiefly as an aid in opening a passageway which, as others have observed, is chiefly between cells.

When such larvae work out through the cut surface of the section, activity of the stylet stops at once, and a single larva seen moving outward from an egg mass through the large intercellular spaces of the open cortical tissue of an old gall, did not use its stylet at all. Apparently, in young larvae, the stylet is thrust chiefly or only when the lips are in contact with something firm.

Nematodes that have assumed their fixed position within the plerome, both before visible enlargement of the body and at various stages up to almost the beginning of egg deposition, have been observed in detail. Characteristically, they exhibit the same types of activities, differing in degree with the stage of the parasite. Except during the ecdyses and while actively feeding, the stylet is never long at rest, thrusting persistently in different directions, as was true of females in nutrient solution. In the earlier stages the head of the parasite lies rather loosely in an intercellular space opened by the rounding up of young giant cells. Within this space the head of the nematode moves back and forth as its lips make contact with one cell after another. Such movements involve bending of the whole neck region. With growth of the parasite and further swelling of the giant cells against the surrounding, hypertrophied tissues of the gall, this space around the head is gradually reduced until none remains visible, the anterior part of the parasite being tightly pressed against the adjacent walls. At this stage the

head still is moved freely and through an even wider angle than earlier, pointing the stylet in all directions, as was true of nematodes in nutrient solution, but associated with such movements there is no perceptible change in the outline of the anterior end of the parasite, which continues at all times to fill tightly the socket between the giant cells. Likewise, at least in pea root, there are no visible spaces between contiguous giant cells near the head. The apparent space within which the head of the mature nematode lies in sections of fixed material is an artifact. It is evident, in live material, that the stylet of the adult female cannot be far protruded in any direction without penetrating the wall of one of the giant cells.

Penetration of such walls by nematodes of various developmental stages has been seen repeatedly, the slender stylet tip passing through the wall and extending well into the cell. Most of the penetrations observed were into giant cells that had been cut in sectioning and that, in consequence, had lost their protoplasm. Stylets penetrating into such cells were withdrawn within a few seconds without pulsation of the esophageal bulb. In a few fortunate cases, however, narrow ends of deep-lying cells that had not been cut into, extended up to the median plane of the nematode so that the stylet tip was visible near the upper surface of the dense protoplast. In such cells the stylet was held for long periods. Sometimes 15 minutes or more elapsed after penetration before pulsation of the bulb began, and pulsation was observed to continue from a few minutes to over an hour. In every instance, pulsation occurred only while the stylet tip was within a giant cell. Many times nematodes were seen feeding with the stylet directed downward. Here the position of its tip was not visible, but by watching the position of the basal swellings of the stylet, it was clearly apparent that the stylet was far protruded, which means, from the nature of the enclosing walls, that its tip was in a cell. In each such instance the cell in question was one which had not evacuated at the time of sectioning. Clearly this nematode feeds, in all stages, with its stylet inserted into cells.

Saliva flow has not been seen under these conditions, but the protoplasm of normal giant cells would obscure it except in a most favorable situation. The usual pause after penetration of a cell before actual feeding begins, as indicated by pulsation of the bulb, suggests that it occurs frequently.

#### DISCUSSION

These observations not only provide a picture of the mechanics of feeding of *Heterodera marioni*, but also are significant in several other respects. With observations on *Dorylaimus*, *Discolaimus*, *Actinolaimus*, *Aphelenchus*, *Aphelenchoides*, *Ditylenchus*, and *Heterodera*, all in agreement, it now seems safe to generalize to the extent that nematodes equipped with hollow, axial stylets (buccal stylets in the tylenchs and odontostylets in the dorylaims)

use them as piercing organs and feed with them protruded into their food substance or organism.

The members of these genera that the writer has thus far seen feeding all feed upon living or freshly killed plants or animals. This justifies the suspicion long held by nematologists that the many free-living nematodes with similar stylets, found associated with the roots of plants, include harmful external parasites the pathogenic significance of which is yet to be determined.

The former view that *Heterodera marioni* secretes into and feeds from intercellular spaces now appears attributable to the limitations of the usual histological techniques as applied to nematodes. After the parasite is killed and fixed within gall tissue, its aspect is greatly changed. Hot fixation often is used with nematode material, and observations by the writer indicate that a nematode killed by heat relaxes so that its stylet retracts and its head swings forward to a symmetrical posture. Slow killing with some cool, diluted fixing solutions has a similar effect, even though a nematode with its stylet protruded may be killed by cool, strong solutions without retraction. Even so, the almost inevitable shrinkage of the nematode during fixation and later processing, would be most likely to pull the stylet out of the cell wall. In this connection the writer has examined some 100 available slides prepared several years ago by different workers and with varied techniques, without finding a single definite instance of a protruded stylet. In every preparation the parasite was shrunken and distorted. Published photomicrographs of other workers indicate that this difficulty is general.

The observation of saliva flow confirms the hypothesis of earlier investigators formulated on the basis of histological investigations, but it is no longer necessary to postulate that this saliva so alters permeability of the giant cells that nutrient substances diffuse outwards into intercellular spaces. Apparently, it is injected into the giant cells and doubtless is in some way the cause of their peculiar development. Since it has now been demonstrated that such saliva may be extruded into a drop of fluid, so that some knowledge of its nature may be obtainable by microchemical methods, the writer refrains from postulating the nature of its action. It is clear, however, that this saliva is less actively proteolytic than that of the predacious species of *Aphelenchoides*, otherwise the injected cells would promptly be killed. Likewise, it is different in appearance, which in itself is an indication of different composition. Within the dorsal duct this saliva appears to consist of minute granules suspended in fluid, while that of the *Aphelenchoides* is much more coarsely globular. The question is raised as to whether saliva of *Heterodera marioni* contains toxic substances that diffuse widely through the host plant, producing a systemic disturbance of functions.

Success in observing the feeding process in nematodes removed from the gall, over a period of five days, suggests that, with an adequate nutrient solu-

tion and aseptic technique, it may be possible to rear this internal parasite *in vitro* and determine its nutritional requirements. Its known host range, exceeding 1,100 species of plants, in itself demonstrates adaptability to varied types of nutrition. Still, the fact that pulsation of the esophageal bulb was seen most commonly in nematodes found with the stylet protruded when removed from the root, justifies the suspicion that some stimulus associated with the penetration of a cell may be a requisite for the initiation of feeding.

It would seem probable from these observations that giant cells are cells that have received injections of saliva and have been fed upon, and thus that all of them have at least some small surface within reach of the stylet. Certain published illustrations give the impression that they do not, although they clearly are all near the head and begin their development adjacent to the head. Determination of this point probably will require careful reconstruction from serial sections. From the writer's observation of thick sections it is very evident, however, that certain cells, which extend far out from the head, have at least a narrow arm within reach of the stylet, and the common forms of giant-cell groups show plainly that many if not all such cells are in immediate contact with the remarkably mobile head.

*Heterodera marioni* feeds by thrusting its slender stylet into a cell adjacent to its head, apparently injecting saliva and then sucking out only part of the cell contents, then retracting its stylet, turning to another cell and repeating the process, and then to another. In such a manner, feeding on all cells within reach of its stylet, it avoids the early destruction of cells and maintains conditions suitable for long continued feeding in a small group of host cells that, stimulated by the action of the parasite, develop to sufficient size and metabolic activity that they can withstand the repeated withdrawal of a part of their substance. The young larva feeds less vigorously and copiously, for its esophageal bulb is smaller and weaker than in adults and its food requirements are less. Giant cell growth appears to keep pace with increasing requirements of the enlarging parasite until, before egg deposition begins, these cells have sufficiently great surfaces of contact with adjacent conductive and parenchymatous cells that an ample and constant supply of nutrient materials is available for the necessarily rapid replacement of their substance persistently withdrawn by the parasite.

#### SUMMARY

The feeding of *Heterodera marioni* has been observed both in nutrient solution and in sections of live galls. Techniques and essential optical equipment are specified.

This nematode obtains its food not by secreting saliva into intercellular spaces and then sucking up nutrient substances exosmosed from adjacent giant cells, but rather by penetrating cells with its slender stylet and feeding directly from their substance.

Both in the root and in solution, this nematode swings its head freely and through wide angles, not only dorsally and ventrally but laterally as well, and it protrudes its stylet freely at any angle. Within the root, the anterior end of the mature female is so firmly encased by the rigid walls of giant cells that swinging of the head is not accompanied by perceptible changes in general outline.

In solution, saliva coming apparently from the broad duct dorsad of the lumen of the esophagus, has been seen to flow outward from the extreme tip of the protruded stylet. This has been seen only in nematodes found with the stylet fully protruded when removed from the root. Saliva flow into a giant cell has not been seen, but circumstances suggest that it does occur, obscured by the dense protoplasts of such cells.

The earlier hypothesis of saliva secretion into an intercellular space at the head of the parasite is invalidated by the absence of such a space in the live, mature specimen. The space seen generally in histological preparations is an artifact, resulting from shrinkage of the nematode.

In a solution of dextrose and peptone, intermittent pulsation of the esophageal bulb has been observed as long as 120 hours after removal from the root, but only while the stylet was far protruded. In root sections mounted in water, pulsation has been seen only when the stylet tip was held protruded into a live giant cell.

The longest observed period of continuous pulsation, slightly exceeding an hour, was insufficient to empty the cell fed upon. Feeding briefly and in irregular rotation upon all the giant cells within reach of its mobile head, this nematode avoids destruction of cells and thus maintains, for a long period, an immediately accessible and abundant food supply.

Significance of these observations is discussed in relationship to other stylet-bearing nematodes and to the biology and pathogenicity of this species.

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# LEGUME VIRUSES IN IDAHO<sup>1</sup>

W. H. PIERCE

(Accepted for publication April 1, 1937)

It recently has been established by Zaumeyer and Wade (6, 7), Pierce (2, 3), Osborn (1), Stubbs (4), and others, that several distinct viruses are involved in the mosaic diseases of various legume crops. Not only has it been shown that individual hosts are subject to infection by several viruses, but also that distinct viruses are often capable of infecting a wide range of leguminous plants. In previous papers (2, 3) it was shown how the various viruses might be identified in the greenhouse on the basis of differential hosts and physical properties.

During the spring and summer seasons of 1935 and 1936, a number of leguminous plants affected with viroses were collected from various sections of Idaho and tested in the greenhouse on differential hosts in an effort to identify the viruses concerned. The results of these tests showed that, while each legume species usually was affected with a virus peculiar to it, there were many natural instances of one species being host to any of several different viruses.

## MATERIALS AND METHODS

Legumes affected with viroses were collected from various sections of Idaho and tested in the University of Idaho greenhouses at Moscow, Idaho,

TABLE 1.—*Reaction of Stringless Refugee Green and Asgrow 40 and Perfection peas to viruses collected from several sections of Idaho and tested in the greenhouse, University of Idaho, Moscow Idaho*

Stringless Refugee Green beans	Asgrow 40 peas	Perfection peas	Virus
Negative	Systemic mosaic with leaf, stipule, and pod enations (Fig. 1, B)	Spotted mosaic with enations (Fig. 1, A)	<i>pea virus 1</i>
Negative	Systemic yellow mosaic (Fig. 1, C)	Negative	<i>pea virus 3</i>
Systemic yellow mosaic (Fig. 2, A)	Systemic mild mosaic (Fig. 1, F)	Negative	<i>bean virus 2</i>
Local necrotic lesions (Fig. 2, D)	Negative	Negative	<i>alfalfa virus 2</i>
Systemic mild mosaic (Fig. 2, C)	Severe mosaic and/or necrosis	Severe mosaic and/or necrosis (Fig. 1, D, E)	<i>white clover virus 1</i>

<sup>1</sup> Published with the approval of the Director of the Idaho Agricultural Experiment Station as Research Paper No. 157.



on stringless Refugee Green beans (*Phaseolus vulgaris* L.), Asgrow 40 peas, and Perfection peas (*Pisum sativum* L.). The viruses were roughly identified by their reaction on these 3 hosts (Table 1).

Further analysis of the viruses was not made except in the case of those viruses classified as *pea virus 3*. Since Stubbs (4) had shown that a similar virus differed in being nontransmissible to red clover, all of the viruses identified as *pea virus 3* were subsequently inoculated to red clover. In all cases, however, positive infection was obtained, indicating that viruses of the type described by Stubbs as *pea virus 2 ABC* were not encountered in this survey.

For a more complete description of the viruses identified in this paper, reference may be made to the descriptions given in two previous papers (2, 3). The legume virus nomenclature followed in this paper is the same as used in previous papers from this laboratory.

All inoculations were made by grinding infective material in a mortar and then straining through cheesecloth. A small amount of carborundum powder was added directly to the inoculum. Test plants were then inoculated by wiping their leaf surfaces with a cheesecloth pad that had been immersed in the inoculum. After inoculation the plants were rinsed with water.

#### RESULTS

A total of 116 legume plants affected with viroses were tested on young disease-free seedlings of Stringless Refugee Green beans, Asgrow 40, and Perfection peas, and the identity of the viruses determined as outlined above. Typical symptoms obtained on these hosts are shown in figures 1 and 2.

##### Tests of Red Clover

As shown in table 2, a total of 28 red-clover, *Trifolium pratense* L., plants were tested. Of these plants, 19 were diagnosed as being affected with the common pea mosaic virus (*pea virus 3*). Five were found infected with the yellow bean mosaic virus (*bean virus 2*). Three were found to produce only local necrotic lesions on Stringless Refugee Green beans, and were, therefore, considered to be infected with *alfalfa virus 2*. And one plant was found affected with white-clover mosaic caused by *white-clover virus 1*. It is apparent from the analysis made on red clover that this host is susceptible to a number of different viruses and that the use of the term red-clover mosaic alone conveys little about the identity of the causal virus.

##### Tests of Sweet Clover

Thirty-two plants of white sweet clover, *Melilotus alba* Desr., and yellow sweet clover, *M. officinalis* (L.) Lam., were tested. Tests from these 2

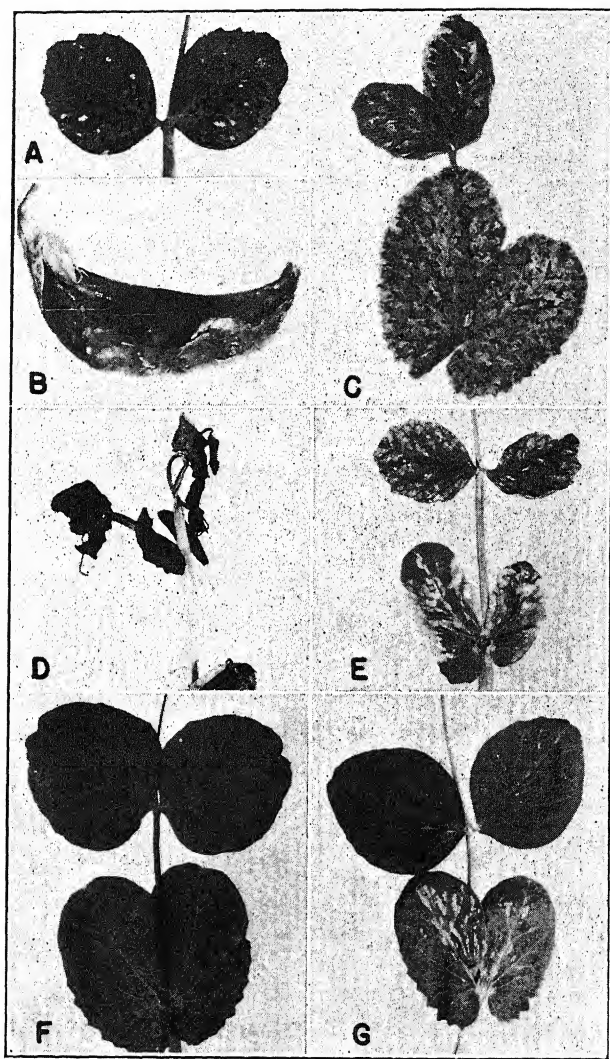


FIG. 1. Symptoms of certain legume viroses on peas. A. Perfection variety showing typical spotting symptom produced by *pea virus 1*. B. Pod of Asgrow 40 variety showing enations and malformation caused by infection with *pea virus 1*. C. Asgrow 40 variety infected with the common pea-mosaic virus (*pea virus 3*). D and E. Perfection variety showing necrosis and severe mosaic following inoculation with *white-clover virus 1*; in some instances complete necrosis takes place, as in (D); in other instances plants are not completely killed but develop severe mosaic, as shown in (E). F. Mild mosaic symptoms on Asgrow 40 due to infection with *bean virus 2*. G. Perfection, noninfected control.

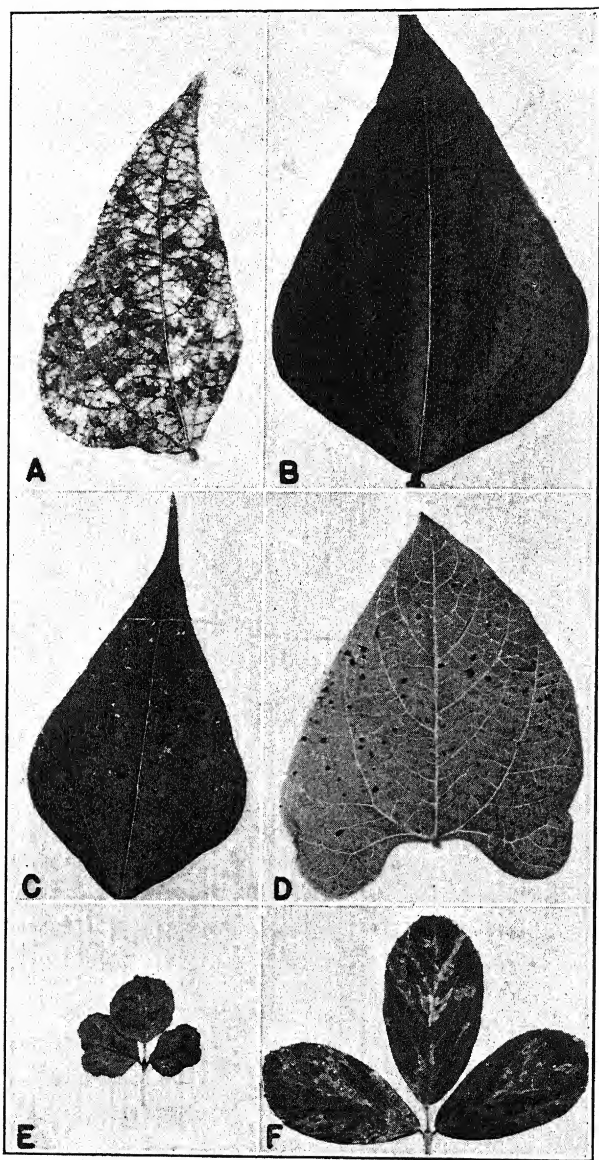


FIG. 2. Leaf symptoms produced by certain legume viruses on Stringless Refugee Green beans and alfalfa. A. Well-developed symptoms of yellow bean mosaic caused by *bean virus 2*. B. Noninoculated control. C. Mild mosaic symptoms on bean following inoculation with *white-clover virus 1*. D. Local necrotic lesions on bean caused by *alfalfa virus 2*. E. Dwarfing, crinkling, and mottling on alfalfa due to infection with *alfalfa virus 2*. F. Alfalfa showing a mosaic, nontransmissible by artificial methods to beans and peas.

species were not kept separate. The yellow bean-mosaic virus (*bean virus 2*) was found to be responsible for the virosis of 29 of these plants. One plant was affected with *pea virus 3*, one with *alfalfa virus 2*, and one with *white-clover virus 1* (Table 2).

TABLE 2.—Results of tests of leguminous plants naturally infected with viroses in Idaho (1935-1936)

Host	Total plants tested	Viruses identified
	<i>Number</i>	
Red clover	28	19 <i>peas virus 3</i> 5 <i>bean virus 2</i> 3 <i>alfalfa virus 2</i> 1 <i>white-clover virus 1</i>
Sweet clover	32	29 <i>bean virus 2</i> 1 <i>pea virus 3</i> 1 <i>alfalfa virus 2</i> 1 <i>white-clover virus 1</i>
Peas	24	2 <i>pea virus 1</i> 20 <i>pea virus 3</i> 2 <i>bean virus 2</i> 2 <i>alfalfa virus 2</i> 2 <i>white-clover virus</i>
Alfalfa	20	16 No infection on peas or beans 2 <i>pea virus 3</i> 3 None
Alsike clover	5	5 <i>white-clover virus 1</i>
White clover	5	2 <i>white-clover virus 1</i>
Yellow trefoil	2	1 <i>pea virus 3</i>
White lupine	1	

#### Tests of Peas

Twenty-four collections of peas, *Pisum sativum* L., affected with mosaic, were tested in this study, and it was found that 20 were affected with the common pea mosaic virus (*pea virus 3*), the most common virus found affecting peas in Idaho. Two plants were infected with the enation type of pea mosaic virus (*pea virus 1*), and 2 with *bean virus 2*.

#### Tests of Alfalfa

Alfalfa, *Medicago sativa* L., plants exhibiting mosaic symptoms often yielded negative results in the tests on beans and peas. In all, a total of 20 alfalfa plants were tested; 16 gave no infection of any kind on peas and beans. Two plants were found infected with *alfalfa virus 2*, and 2 with the type of white-clover virus described by Zaumeyer and Wade (6), which differed slightly from *white-clover virus 1* in that it produced distinct local lesions, as well as systemic infection on Stringless Refugee Green beans.

Weimer (5) described an alfalfa-mosaic virus that he was unable to transmit artificially but that could be transmitted by aphids. It would appear that the virus encountered in this study and that failed to give positive infec-

tion may have been the same as Weimer's alfalfa virus. Zaumeyer and Wade (6), however, tested an alfalfa virus obtained from Weimer and found it to produce local lesions on bean and, therefore, to be the same as, or similar to, *alfalfa virus 2*. Regardless of the identity of Weimer's (5) alfalfa virus, it seems apparent that there is an alfalfa virus that differs from *alfalfa virus 2* in being nontransmissible to bean and peas by present artificial methods. In figure 2, F, are shown alfalfa leaflets with symptoms similar to those depicted by Weimer (5). This is the type of alfalfa mosaic commonly encountered in this investigation and that proved to be nontransmissible to peas and beans. In figure 2, E, are shown alfalfa leaflets infected with *alfalfa virus 2*. The latter virus usually produces considerable stunting and malformation, but this cannot be relied upon for accurate diagnosis. It is preferable to determine the identity of these viruses by their reaction on beans.

#### Tests of White Clover

Only 5 plants of white clover, *Trifolium repens* L., were tested, but in each case the white-clover-mosaic virus (*white-clover virus 1*) was obtained. This is what might have been expected, since it was shown in previous papers (2, 3) that white clover was susceptible to *white-clover virus 1* and *alfalfa virus 2* only of the viruses used.

#### Tests of Miscellaneous Plants

Five plants of alsike clover, *Trifolium hybridum* L., showed 2 infections with *pea virus 3*. The 3 other plants tested gave negative results.

Tests with 2 plants of yellow trefoil, *Medicago lupulina* L., showed both plants to be infected with the white-clover-mosaic virus (*white-clover virus 1*).

An experimental planting of white lupines, *Lupinus albus* L., was found infected with the common pea-mosaic virus (*pea virus 3*).

#### Virus Infections of Beans

No greenhouse tests were made in this investigation to determine the identity of the virus infections occurring on beans, *Phaseolus vulgaris* L., since these viruses had been previously (2, 3) differentiated and could be fairly accurately determined by inspection. In order to indicate here the relative percentages of the various types of virus infection occurring on beans, data from trial bean plantings at Twin Falls, Idaho, in 1935 and 1936 are presented in table 3. The percentages of curly top (sugar-beet curly-top virus), common bean mosaic (*bean virus 1*), and yellow bean mosaic (*bean virus 2*) are given for 4 varieties of beans. Two varieties, Great Northern U.I. No. 123 and Idaho Refugee, are completely resistant to common bean mosaic.

TABLE 3.—Percentages of the different types of virus infections found occurring in experimental plantings of beans at Twin Falls, Idaho, in 1935 and 1936

Variety	Curly top		Common bean mosaic ( <i>bean virus 1</i> )		Yellow bean mosaic ( <i>bean virus 2</i> )	
	1935	1936	1935	1936	1935	1936
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Common Great Northern .....	9	1	85	18	— <sup>a</sup>	— <sup>a</sup>
Great Northern UI 123 .....	5	.03	0	0	2	2
US No. 1 Snap Bean .....	51	15	17	11	— <sup>a</sup>	— <sup>a</sup>
Idaho Refugee .....	24	2	0	0	4	1

<sup>a</sup> Not determined because of confusion with common bean mosaic.

### Conclusions and Summary

The viruses dealt with in this paper were for the most part those (2, 3) previously described and differentiated as viruses primarily affecting leguminous hosts. It is here considered unnecessary to repeat how each virus was originally identified.

The system used in this paper did not take into account minor variations in the viruses encountered. As shown in table 1 only 3 hosts were used. It is readily admitted that the use of additional hosts probably would have resulted in the differentiation of a larger number of viruses or virus strains. Variations in virulence and in symptom expression were often evident among viruses classified as the same virus. Thus, among the viruses identified as *bean virus 2*, some were definitely more severe in their effects on beans than others.

As shown in table 2, most legume hosts were subject to more than one virus, thus emphasizing the fallacy of referring to red-clover mosaic or other legume mosaics without specifying the particular virus involved. On the other hand, it was found that one virus generally predominated in the infections on each species. Thus, the majority of infections on red clover were due to *pea virus 3*, and those on sweet clover, to *bean virus 2*. It is conceivable, however, that in certain geographic locations other viruses might predominate on individual species. It seems essential, therefore, in referring to legume mosaics to include the identity of the virus or viruses concerned.

Of significance in this investigation is the demonstration that certain leguminous plants may serve as overwintering hosts of certain viruses that attack annual crops. Thus, sweet clover was found to overwinter *bean virus 2*, which causes yellow mosaic of beans. Likewise, red clover undoubtedly serves as an important overwintering host of *pea virus 3*, the cause of common mosaic of peas. These relationships also have been shown to exist in a number of other sections of the United States by Zaumeyer and Wade

(6, 7). No host other than peas was found for the enation pea-mosaic virus (*pea virus 1*). This is not particularly surprising, since only 2 cases of enation mosaic were found on peas in Idaho. It is probable that in regions where this virus is prevalent, overwintering hosts may be found.

Of the viruses found to occur on peas and beans in Idaho, the most important from an economic standpoint appear to be the common pea-mosaic virus (*pea virus 3*), which is severe on green pod peas in certain sections, the common bean-mosaic virus (*bean virus 1*) on susceptible varieties of beans, and the sugar-beet curly-top virus, which may completely destroy stands of susceptible beans in years when the beet leaf hopper is prevalent.

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# CROWN GALL ON INCENSE CEDAR, *LIBOCEDRUS DECURRENS*<sup>1</sup>

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## INTRODUCTION

Only recently have galls produced by *Pseudomonas tumefaciens* Smith and Townsend been reported in the literature on the conifers. The species on which the galls have been investigated are: *Juniperus sabini* L. (p. 133<sup>2</sup>), *Cupressus arizonica* Green (2), *Sequoia gigantea* (Lindl.) Dec. (9), *Araucaria bidwillii* Hook. (11), *Libocedrus decurrens* Torr. (10), and a gall<sup>3</sup> of bacterial origin, but probably not caused by *Ps. tumefaciens*, on *Pseudotsuga taxifolia* (Lamb.) Britt. (3). The Arizona Experiment Station (1) reports successful isolations of *Ps. tumefaciens* from *L. decurrens* sent from California, and successful inoculation with strains of the crown-gall organism isolated from cottonwood and *L. decurrens*.

## MATERIAL AND ISOLATION

About 1910, W. T. Horne, of the Division of Plant Pathology, University of California, had growing on his Berkeley lot an incense cedar 6 feet in height, which had typical crown galls 3-4 inches in diameter at the base of the trunk.

In 1916, specimens of galls on incense cedar, *Libocedrus decurrens*, were sent to the author from the Department of Plant Pathology, University of California, Berkeley. The galls were on nursery trees received as small seedlings from the United States Forestry Station near Quincy, California, and grown by the Division of Forestry, University of California.

The trees had been inspected by the Division of Forestry when received and again when planted in nursery rows in March, 1916, and nothing abnormal was detected. When the trees were taken up the following December, 10 per cent of them showed galls (Fig. 1). Different species of pine and the Douglas fir, planted in adjacent nursery rows, were free from galls. The percentage of crown gall listed above is much greater than is usually found in seed beds and nurseries of *Libocedrus decurrens*. Gall infection does sometimes appear in negligible amounts of a few seedlings to as much as 2.5 per cent, is apparently greater on land recently cleared of the native nonconifer-

<sup>1</sup> Paper No. 369, University of California Citrus Experiment Station and Graduate School of Tropical Agriculture, Riverside, California.

<sup>2</sup> Division of Mycology and Disease Survey. Diseases of trees. U. S. Dept. Agr. Bur. Plant Indus. Plant Disease Repr. Sup. 81: 132-135. [Mimeographed.]

<sup>3</sup> Hansen, H. N., and Ralph E. Smith. A bacterial gall disease of Douglas fir, *Pseudotsuga taxifolia*. Hilgardia 10: 569-577. 1937.



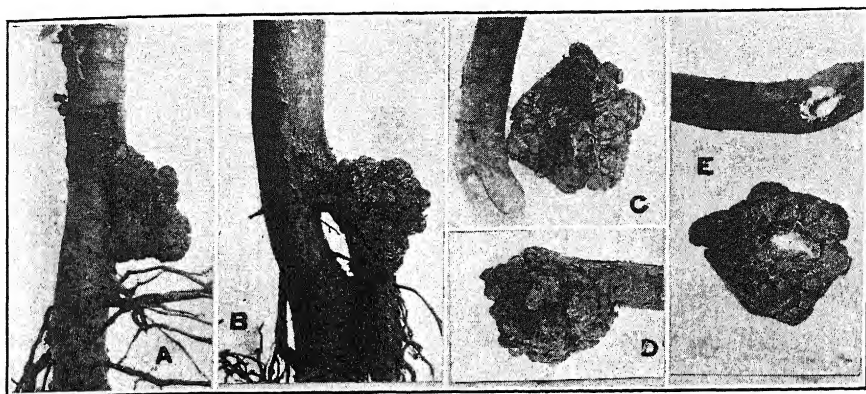


FIG. 1. Natural galls on *Libocedrus decurrens*. A. A superficial gall development. B. A gall on a root. C-E. Views of the same gall, which is dissected in C and E to show the small point of attachment represented by the white area.

ous growth, and might become troublesome on ground previously infected by other plants.

The crown-gall organism was isolated from the galls of *Libocedrus decurrens* in December, 1916, and this may have been the first time that *Pseudomonas tumefaciens* was secured from a gall on a conifer. The results of the investigation were not published, but subcultures were furnished to other investigators and the results from their use are reported in the literature.

The incense cedar culture was used by Patel (7) as one of the strains in his agglutination studies, and by Muncie and Patel (6), in their study of a bacteriophage for *Pseudomonas tumefaciens*. Riker and his associates (8, Table 3, p. 518) produced galls with the incense-cedar culture on tomato and tobacco but not on apple. Hendrickson, Baldwin, and Riker (4, pp. 598, 614, Table 1) used cultures from single-cell progenies of this original incense-cedar culture in their study of variations of bacteriological characteristics in different strains of the crown-gall organism.

After all these years of growth on artificial media, the cultures from incense cedar used in making the inoculations are still pathogenic on tomato, but their virulence on the incense cedar is apparently less than that of the more recently isolated cultures from peach and willow. The incense-cedar culture has been passed at least twice through the tomato and has been reisolated, and it is these cultures that were used in some of the inoculations. Cultures from peach and willow also were used.

The outgrowths on incense cedar from which the causal organism was isolated were 20 to 30 mm. in diameter. They were rough, irregular in shape, and superficial. They had formed much growth over the normal bark tissue and some of them had a small point of attachment at the base (Fig. 1, C



FIG. 2. Artificial galls produced on different hosts by the incense-cedar organism. A. *Schinus molle*, photographed after 1 year. B. Tomato after 70 days. C. *Cupressus sempervirens*, after 1 year. D. *Diospyros kaki*, after 80 days.

and E). They were not united with the bark, except in one small area, but had made a growth over the surface of healthy bark tissue. In general, the

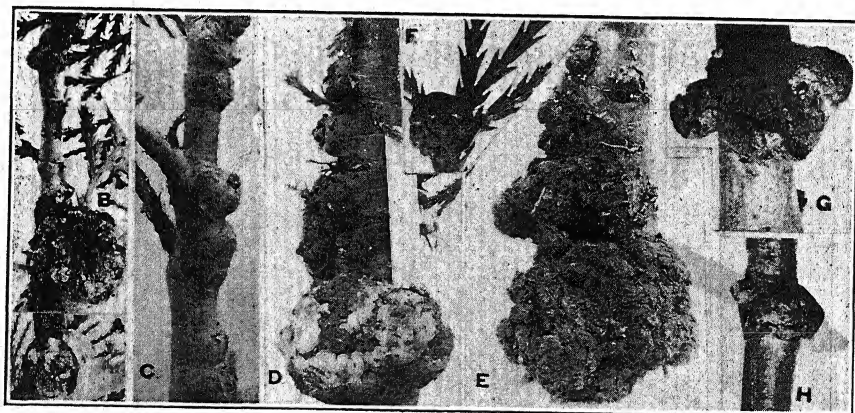


FIG. 3. Artificial galls produced on *Libocedrus decurrens* by the crown-gall organism isolated from peach. A. Gall on a small twig 10 months after inoculation. Observe the crack caused by the tension of tissue and the growth of a small hypertrophy near the center. B. The same inoculation 14 months after inoculation. The gall near the base is 20 mm. in diameter. C. Inoculations showing different forms of tissue response. A small gall has formed on one of the healed-over, enlarged areas. Later observations showed further increases in growth. D. Gall produced at the base of the trunk of a small seedling 3 months after inoculation. E. The same gall 10 months after inoculation. Note the superficial gall-like formation above the globose gall in D and E. Gall on a small twig after 20 months. G and H. Views of different-size galls resulting from a series of five inoculations of which two were negative.

galls were much like crown gall as it appears on other hosts, but the shape was somewhat abnormal. This superficial gall development appeared on the artificial inoculation (Fig. 3, D).

#### ARTIFICIAL INOCULATIONS AND RESULTING GALLS

In the earlier studies the inoculations with the incense-cedar culture were made on several different hosts that are susceptible to crown gall, but, because of lack of material, not on the incense cedar. These results are summarized in table 1. Galls were formed by inoculations on tomato, *Salix* sp., *Prunus*

TABLE 1.—Summary of inoculations on different species of plants with cultures of *Pseudomonas tumefaciens* isolated from incense cedar, *Libocedrus decurrens*

Species inoculated	Date of inoculation	Number of inoculations	Number of galls	Diameter (mm.)
<i>Cupressus sempervirens</i> .....	Aug. 7, 1917	5	4	5-10
<i>Cupressus macrocarpa</i> .....	Aug. 13, 1917	10	0	
<i>Salix</i> sp. ....	Feb. 19, 1917	10	3	15-20
<i>Prunus cerasifera</i> .....	Feb. 30, 1917	10	6	10-15
Tomato .....	Feb. 24, 1924	10	Positive	5-10
<i>Schinus molle</i> .....	Feb. 24, 1933	10	6	15-30
<i>Diospyros kaki</i> .....	May 14, 1936	40	12	5-20
Tomato .....	May 13, 1936	5	3	10-15

*cerasifera*, *Schinus molle* (pepper tree), *Cupressus sempervirens* (Italian cypress), and *Diospyros kaki* (persimmon). These galls on *Cupressus sempervirens*, made in 1917 with the incense-cedar culture, were possibly the first to have been produced artificially on a conifer. Galls on 4 of the above hosts are illustrated in figure 2. Galls also were produced on *Libocedrus decurrens* by artificial inoculations with a culture of the organism from peach.

TABLE 2.—Artificial inoculations on *Libocedrus decurrens* by *Pseudomonas tumefaciens* in series of five (data taken May 16, 1936)

Source of culture	Date of inoculations	Number of inoculations	Number of galls	Diameter (mm.)
Incense cedar .....	Sept. 12, 1934	20	0	0
Peach .....	Sept. 13, 1934	10	1	2
Peach .....	Nov. 2, 1934	5	0	.....
Incense cedar .....	Dec. 7, 1934	5	0	.....
Peach .....	Mar. 9, 1935	5	0	.....
Peach .....	Apr. 26, 1935	15	12	3- 5
Incense cedar .....	Apr. 27, 1935	15	2	3- 5
Peach .....	June 8, 1935	15	6	10-25
Peach .....	July 5, 1935	15	4	5-10
Peach .....	July 10, 1935	15	4	5
Peach .....	July 25, 1935	5	0	.....
Incense cedar .....	Apr.-July, 1936	15	4	2- 3
Incense cedar .....	Aug. 10, 1936	1	1	17

In table 2 are presented the results of more recent inoculations with different crown-gall cultures on incense cedar.

The aerial overgrowths induced in incense cedar show much variation in shape. At least two types of overgrowth can be found. The more typical crown gall (Fig. 3, D) was at the base of a small incense cedar tree and was developed in a marcot box where conditions were moist. After the growth of this gall had continued for a time and had become dried out, it assumed the more typical shape of crown gall (Fig. 3, E). The more usual form of crown gall was developed on a young twig (Fig. 3, F). In the second type, the wounds of the inoculated tissue appear to heal over at first (Fig. 3, C), but in a few months this tissue may become swollen and somewhat globose and may be 5 to 15 mm. in diameter. The overgrowth appears to have a normal bark and the tissue is firm like normal tissue.

Often from this enlarged tissue occurs a secondary growth having the appearance of gall tissue or the beginning stage of crown gall. This growth may persist for a time but usually corks off and disappears (Fig. 3, C).

Sometimes the globose structure may crack and from the interior may develop an abnormal growth resembling the beginnings of crown gall (Fig. 3, A), which with time may become a typical gall (Fig. 3, B). Also, on these aerial enlargements a small growth of gall tissue may show the early stages of development, but usually it does not increase in size and become a typical crown gall. Point-like or papillate projections may appear near the point of inoculation and sometimes from the enlarged tissue (Fig. 3, G and H). Levine (5, p. 188) has reported what seems to be similar plant responses, the development of papillae and small swellings of tissue at the points of inoculations with a virulent culture of *Pseudomonas tumefaciens* on sunflower and Ricinus. While there appears to be a definite tissue reaction after inoculation, the explanation of what actually takes place will require further study. Puncture wounds as controls for the inoculations were made. These injuries always healed in a normal manner and with no indication of abnormal growth.

#### REISOLATION OF CAUSAL ORGANISM

The crown-gall organism often is reisolated with difficulty from woody aerial galls, and this was true of the artificial galls on incense cedar. Reisolations were unsuccessful from some of the best available material; these galls are illustrated in Fig. 3, D, E, F, and G. After the dead gall tissue was removed from the gall shown in Fig. 3, E, fresh hypertrophies developed on the old lesion kept in a marcot box, and from this material the causal organism was recovered and its pathogenicity demonstrated on tomato.

#### SUMMARY

The crown-gall organism has been isolated from galls on *Libocedrus decurrens*. This culture caused typical crown gall on other hosts: Tomato, tobacco,

*Salix* sp. *Prunus cerasifera*, and *Cupressus sempervirens*. Galls were produced artificially on *L. decurrens* with cultures from incense cedar, peach, and *Salix* sp. Some of these artificial galls on *L. decurrens* are not entirely typical of crown gall as it often develops on susceptible hosts. *L. decurrens*, when growing under cultivation, has been susceptible to crown gall.

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# INFECTIVITY OF THE FIRE-BLIGHT ORGANISM

E. M. HILDEBRAND

(Accepted for publication May 6, 1937)

The number of bacteria (*Erwinia amylovora*) required to initiate infection in fruit trees is of interest, not only because of its bearing on the relationships between pathogen and host (or suspect), but, also, because it throws light on the practical question of dissemination of the bacteria in the spring. Although relatively few bacteria carried into the beehive ever get out again because of the treatment the nectar receives from the bees (Hildebrand and Phillips, 3), the potentiality of dissemination by contaminated bees in their visits to fruit blossoms is enormous. An evaluation of this point will largely depend, however, on the infectivity of these bacteria, or, in other words, on the minimal numbers required to produce the disease.

## EXPERIMENTAL METHODS AND RESULTS

One approach to the infectivity of the fire-blight organism was made through use of the dilution technic based on agar-plate counts and employment of green Kieffer pear fruits as test material. The pear fruits were surface-sterilized with bichloride of mercury 1 to 1000 before using. Four slices were obtained from each fruit and placed cut side up on a disinfected filter paper in a moist chamber. Measured amounts of an average of 12 different bacterial dilutions were placed on the cut surface of 10 slices and the numbers of bacteria estimated by a parallel series of plate counts on nutrient agar. Infection of 2 or more slices out of the 10 from a given dilution was considered positive. Although symptoms began to appear after 2 days, final observations were made at the end of 10 days to allow for the slower disease development in those dilutions approximating minimal concentrations. The minimal numbers of bacteria for infection based on the plate counts ranged all the way from 2 to over 125 in 7 series of experiments. Such variable results, while suggestive, were not fully satisfactory.

Since single cells of the fire-blight organism have been isolated by micropipette technic and grown with relative ease on nutrient media by the writer,<sup>1</sup> another more precise approach was afforded. The isolation technique was similar to that used by Wright and McCoy (4) and Wright and Nakajima (5) until the bacterium was picked up with the micropipette. At this point the pipette was removed from the holder and held in the hand while expelling the bacterium into the infection court. Blowing on the pipette caused a small bead of fluid containing the organism to form at the opening

<sup>1</sup> In manuscript.

and the transfer was made under a binocular dissecting microscope. Before making the next transfer the contents of the pipette were examined in order to be sure the organism had been expelled. With the aid of this technic approximately 100 single cells between 8 and 16 hours of age were transferred to infection courts (stigmas, anthers, nectaries, and wounded nectaries) of pear flowers on dwarf trees forced into bloom in the greenhouse in February and March, but without success in producing infections. Under the same conditions, using dwarf apple trees, infection resulted in 1 out of 2 trials when a single cell was transferred to the nectary. It appears, therefore, that under the rather low relative humidities (about 50 per cent) prevailing in the greenhouse at that time of year, single cells were unable to infect pear flowers. The apple flower became infected probably because, having a closed nectary, the infection court was less subject to desiccating influences.

Because of the difficulties experienced in working with flowers on the trees, further tests were made with excised flowers held in moist chambers in a room at about 24° C. In one series of 15 single-cell inoculations into apple nectaries 9, or 60 per cent, of the flowers became infected. In 3 out of 5 cases with 2 cells, in 4 out of 5 cases with 5 cells and in all cases where 10 or more cells were transferred, infection resulted. All 3 controls, consisting of single-cell transfers made to nutrient broth, grew. After transfer, between 1 and 2 days were required by single cells for good growth in nutrient broth. At 2 days, clouding of the nectar in the inoculated flowers was also evident and blossom infection was under way. In 2 similar experiments with pear flowers the infections were obscured by contaminations. These results indicate that apple nectar acted as a culture medium, which hypothesis was next tested.

Working under approximately aseptic conditions nectar from apple blossoms, withdrawn by a micropipette and deposited on cover-slips, proved a favorable medium for the growth of single cells. Here, growth was obtained in 4 out of 9 transfers and the identity of the cultures was proved by pathogenicity tests on both pear shoots and fruits.

The number of bacteria required to initiate infections in fruits and shoots presents another problem. Under orchard conditions infections occur through both stomates and wounds, but, because of practical difficulties, only extracted juice was used here.

Juice was extracted from succulent shoots and green fruits of pear, sterilized by passage through a Berkefeld filter, and tested as a growth medium in deep-well culture slides. When single cells were employed growth was not obtained in any case. However, fruit juice proved favorable as a culture medium with larger numbers of bacteria, growth being obtained in 20 out of 22 cases. The number of cells producing growth varied from

4 to 100. The 2 cases where growth failed involved 20 and 25 bacteria. Growth was obtained in all 6 cases where 4 or 5 bacteria were used, probably because of the greater facility in selecting and handling small numbers. In juice from shoots, employing similar numbers of bacteria, growth was obtained in only 2 out of 22 cases or when approximately 75 and 100 cells were transferred. The reason for fruit juice being so favorable as a growth medium is beyond the scope of this paper, but Ark (1) offers evidence that it is because of the asparagin content.

#### DISCUSSION AND CONCLUSION

This study supports the view that the inoculum from a single active fire-blight canker might result in a severe epiphytotic (epidemic) of blossom blight in an orchard. To start things off, rain or insects (flies, according to Parker (3)) may be responsible for carrying primary inoculum from the oozing cankers to the flowers. A 25-year-old apple tree in 100 per cent bloom would provide approximately 20,000 spurs or 100,000 blossoms. Assuming a generation time of one hour for the bacteria, it would require about 17 hours under favorable environmental conditions for one cell to produce 100,000 bacteria, potential inoculum for every flower. One bee picking up contaminated nectar may easily visit and infest flowers in 10 other trees on one trip and makes many trips a day. How long the bacteria from a given flower remain on a bee is not known, but, according to Hildebrand and Phillips (2), honey-bees feeding on infested food are still contaminated about a day afterward. With 27 trees to the acre, thousands of bees to the hive, and 4 or 5 days of good weather for insect activity during bloom, a fire-blight epiphytotic might well be expected.

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## ISOLATIONS MADE FROM HEART ROT OF BANANA IN HONDURAS

OTTO A. REINKING

(Accepted for publication May 20, 1937)

Heart rot of banana has been reported from the Philippines (5, p. 220), Trinidad (7) and from Beyrouth, Syria (1). A similar rot occurs on Manila hemp or abaca, *Musa textilis*, in the Philippines (3, 4, 5, pp. 221-222). The disease is reported to be more prevalent on weakened banana and abaca plants. The fungus causing or associated with these troubles has been identified as *Fusarium moniliforme* var. *subglutinans*. A bacterium, apparently, is associated with advanced cases of infection.

Diseased plants are characterized by the rotting of the central group of rolled young leaves. Rotting usually starts at the tip and advances downward until the young central portion of the plant is attacked. The diseased portion is at first yellowed, then turns brown and rots. A bad odor frequently accompanies the rot in advanced cases, undoubtedly due to secondary bacterial decay. Frequently, the central group of softened diseased leaves near the tip is pushed upward in a folded mass. In early stages the disease is confined to the young central heart and does not penetrate into the surrounding older sheaths. In advanced stages the entire central portion may be diseased and the upper part of the plant may die.

During the month of February, 1936, a severe form of the above described heart rot was prevalent in cut-over banana plantations in Honduras, Central America. The central group of young leaves developing from the cut-off plants and from newly developed suckers was severely affected. New growth develops rapidly from cut-down plants, and injury often results to the central group of leaves because of this rapid pushing out of the newly forming leaves. This condition apparently was conducive to infection by the organism concerned.

The following isolations from diseased plants were made: 4 isolations from young cases of infection. Isolations made from a rot of the tip and youngest leaf within the rolled up group; 3 isolations from older diseased cases of infection. Central group of leaves badly rotted; 1 isolation from an older infected leaf surrounding the young infected leaves.

A pure culture of a *Fusarium*, along with bacteria, was isolated from the 8 different diseased plants. Final examination made on January 25, 1937, of the *Fusaria*, showed that all were *F. moniliforme*. Good chain production of microconidia was present in each culture. No inoculation experiments have been conducted to prove the pathogenicity of the organism isolated from the affected plants in Honduras.

Inoculation tests conducted by Foëx and Lansade on banana (1) indicated that a *Fusarium* isolated from a diseased plant from Syria was weakly parasitic, and that a bacterium apparently was the real cause. The heart rot or tip rot of banana described from Trinidad (7), apparently was caused by the same fungus jointly with a bacterium. Infection studies conducted on abaca in the Philippines (3, 4) with a similar *Fusarium* showed that the fungus was parasitic, especially on weakened plants. In these cases, on banana and abaca, the fungus isolated was identified as *F. moniliforme* var. *subglutinans*.

In view of the fact that *Fusarium moniliforme* and *F. moniliforme* v. *subglutinans* have been found associated with or reported as causing similar diseases, it appears that further studies are necessary in order to clarify the situation. It seems that further culture studies are desirable for a final settlement of the identity of the *Fusarium* associated with the disease. In Honduras the fungus isolated was distinctly *F. moniliforme*, while in the Philippines, Trinidad, and Syria, the fungus isolated has been referred to as *F. moniliforme* v. *subglutinans*.

*Fusarium moniliforme* v. *subglutinans* has been reported as being rather widespread in tropical countries, being first found on decaying leaves and pseudostem, in the vascular bundles and exterior of living pseudostem of bananas and in the air in Honduras (6). In addition to the heart-rot cases cited above, it also has been isolated from the tip rot of immature Cavendish bananas in Trinidad (7).

*Fusarium moniliforme* was isolated from corn in Honduras, and also from banana leaves and in the interior of diseased pseudostems and on dead floral parts of banana (6). It has been referred to as the cause of a tip rot of banana fruits in Uganda, Africa (2).

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## A BACTERIAL DISEASE OF DELPHINIUM

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(Accepted for publication April 28, 1937)

In the summer of 1935, a stem rot of *Delphinium ajacis* L. appeared in The New York Botanical Garden, and was brought to the attention of B. O. Dodge,<sup>1</sup> who published an excellent description of the trouble, but beyond this only went so far as to state that it was clearly of bacterial origin. From this point I was requested by Dr. Dodge to continue the investigation.

### DESCRIPTION OF THE DISEASE

The stem decay commonly shows itself in a blackening of the top or blossom end of the stem, from which it extends downward. When cut open lengthwise there is a blackening at the cortex extending into the pith. The pith is softened, moist, and more or less destroyed through decay. The decaying tissues contain bacteria in large numbers. The disease is a parenchyma rot, closely resembling the blackleg of potato caused by *Erwinia phytophthora* (Appel) Holland 1920. In common with the latter, the present organism produces a cytolytic enzyme (pectinase), which dissolves the middle lamella of the cell wall and produces a dissolution of parenchymatous tissue. The vascular elements remain intact. The bacteria occupy the intercellular spaces, and do not invade the cells unless the walls are ruptured. Inoculation of the organism into raw potato slabs shows it to be without action on starch. No spots were found on the leaves to indicate direct stomatal infection.

### TECHNIQUE

To isolate the organism associated with the stem rot, beef-peptone agar plates were made. These gave numerous colonies of one type. Transfers from typical, widely separated colonies were made onto agar, followed by 2 additional replatings and transfers. This procedure gave a pure culture of the organism in question.

With such a pure culture, inoculations were made into healthy plants of the same species, grown in pots, by stem punctures. In all cases a characteristic stem decay followed. Cultures were in each case made from the inoculated plants, and the same organism was recovered (Fig. 1). The salient characteristics of these cultures are as shown in the text immediately following table 1. The group numbers are in accordance with the Chart of The Society of American Bacteriologists, December 30, 1929.

<sup>1</sup> Dodge, B. O. A bacterial disease of *Delphinium ajacis*. Jour. New York Bot. Gard. 36: 257-260. 2 figs. 1935.

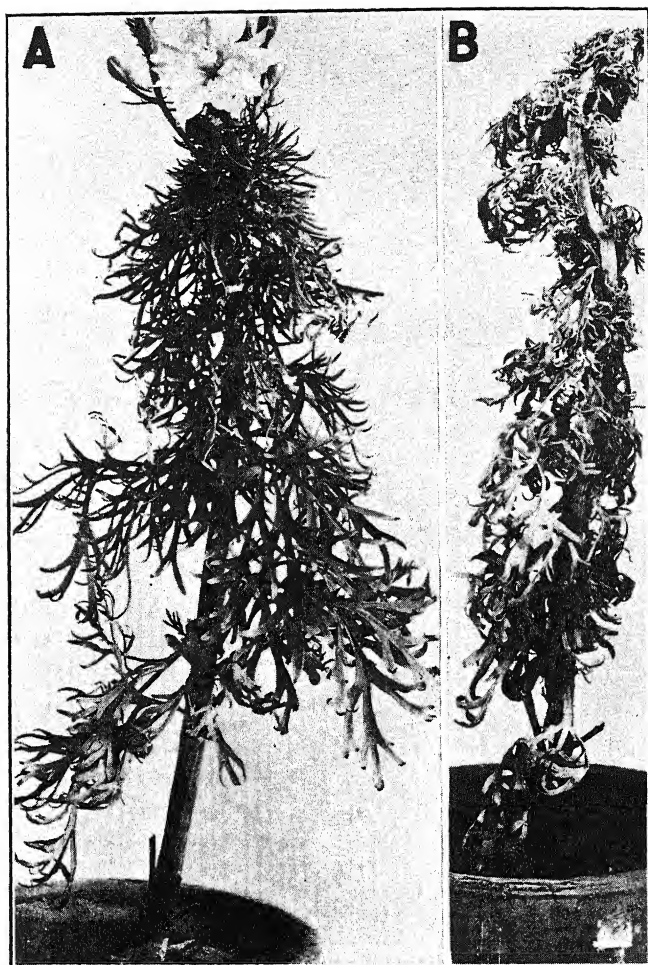


FIG. 1. A. Healthy plant of *Delphinium*. B. Inoculated plant.

TABLE 1.—Culture number, source of culture, and group number of bacteria isolated from naturally and artificially infected plants of *Delphinium ajacis*

Culture no.	Source of culture	Group number	
BD-4 .....	Natural infection, tip	5010.32020.0111.2(1-2)00.	111.21.1000
BD-37 .....	Natural infection, base of stem	5010.32020.0111.2(1-2)00.	111.21.1000
BD-38 .....	From inoculated plant that had been inoculated with macerated tissue from which cult. BD-4 was made	5010.32020.0111.2(1-2)00.	111.21.1000
BD-33 .....	From plant inoculation with BD-4	5010.32020.0111.2(1-2)00.	111.21.1000
BD-32 .....	From plant inoculation with BD-4	5010.32020.0111.2(1-2)00.	111.21.1000
BD-40 .....	From plant inoculation with BD-4	5010.32020.0111.2(1-2)00.	111.21.1000
BD-41 .....	From plant inoculation with BD-4	5010.32020.0111.2(1-2)00.	111.21.1000

## CHARACTERISTICS OF THE ORGANISM

- Morphology.* All cultures were morphologically identical. Rods range from those scarcely longer than broad to short rods, and average  $0.5-0.8 \times 0.8 \times 0.8-1.5$  microns. Occur singly and in pairs. Stain readily with the usual dyes and are Gram negative. Motility more or less pronounced, flagellae peritrichous. No capsules demonstrated. No spores.
- Beef-peptone-agar Colonies* (pH 6.8). Identical in all cultures. Deep colonies, 24 hrs.  $30^{\circ}\text{C}$ .; circular-naviculate, brownish, opaque, borders entire. Surface colonies 48 hrs. 1-2 mm. convex-flat, moist, glistening, borders entire—undulate—notched; centers granular, borders clear, amorphous.
- Beef-peptone-gelatin Colonies.* Surface colonies 4d, not over 1 mm. Microscopically circular, borders sharp, entire. Centers brownish, opaque or finely granular. Colonies alike in all series.
- Gelatin Stab.* 6d, very little growth in depth; surface growth very slowly spreading, After 14-21 days no liquefaction or only a small dry pit.
- Beef-peptone-agar Slant* (pH 6.8). 2-4d, greyish-white, slimy, butyrous, spreading, translucent, glistening. Medium not discolored.
- Beef-peptone Broth.* A uniform persistent turbidity, no surface growth, flocculent sediment. Medium becomes more alkaline.
- Potato.* 4d, growth moist, smooth, glistening, spreading. At first, color of potato, becoming yellowish brown. Flooded with dilute iodine solution, no blue color, but a deep red, due to the formation of erythrodextrin. No reaction for invert sugar in culture or noninoculated plugs.
- Litmus Milk.* Showed coagulation only after 18-20 days, acid. Litmus reduced. No liquefaction of the casein.
- Dunham, Peptone Broth.* Uniform turbidity. Indol not produced.
- Cohn's Solution.* A very faint turbidity.

## PHYSIOLOGICAL CHARACTERISTICS

*Facultative Anaerobic.* Acid and gas from dextrose, lactose (feeble), sucrose, levulose, raffinose, mannitol, arabinose, xylose, salicin, with growth in closed arm. Acid without gas from isodulcitol and glycerose. Inulin and starch not fermented. Ammonia from asparagin, none from peptone. Indol negative or trace only.  $\text{H}_2\text{S}$  negative.  $\text{NH}_3$  negative or traces only in broth. Invertose negative. Diastase negative.

In synthetic media containing  $\text{MgSO}_4 \cdot \text{K}_2\text{HPO}_4$ ,  $\text{CaCl}_2$ ,  $\text{NaCl}$  and  $\text{FeCl}_3$ , with dextrose as a source of carbon, can utilize  $\text{NH}_4$  and  $\text{NO}_3$  as sources of nitrogen.

With  $\text{NH}_4\text{NO}_3$  as a source of nitrogen, with different sources of carbon, produce acid from dextrose, lactose, sucrose, arabinose, xylose, raffinose, salicin, isodulcitol, glycerose, ethyl, and butyl alcohol. No acid from inulin and starch.

In synthetic media containing valine and glycocol as sources of both nitrogen and carbon, growth with  $\text{NH}_3$  production from valine. No growth with glycocol.

## THE QUESTION OF SPECIES

Comparison of the present organism with two strains of *Erwinia phytophthora*, one of which was tested for pathogenicity on potato stems, showed no essential difference in their cultural and physiological characteristics. It should be noted that with the delphinium organism there was no liquefaction of gelatin in 14-21 days, which is contrary to the description of *Erwinia phytophthora* in Bergey's Manual, where this species is recorded as a rather

rapid liquefier. However, in the cultures of *E. phytophthora* tested in parallel with the delphinium organism, it gave also no liquefaction in the same period. Furthermore, records show that liquefaction of gelatin is a somewhat variable characteristic among different strains of the same species, and, therefore, inadequate in species differentiations. We are consequently inclined to consider the delphinium organism as identical with *Erwinia phytophthora*.

#### SUMMARY

A stem rot of *Delphinium ajacis* is described, which is produced by a bacterium identified as *Erwinia phytophthora*. This organism has been isolated from the decaying stems and its characteristics determined. Inoculations of this organism into healthy delphinium plants gave positive infections, and from which the same organism was recovered.

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#### PHYTOPATHOLOGICAL NOTES

*Injury to Cabbage by Lightning.*—The discharge of lightning in cabbage fields in midsummer commonly leads to the destruction of plants in roughly circular spots. Such areas are most commonly encountered some weeks after injury has occurred and to the casual observer the nature of the damage is not always clear. On several occasions the writer has studied the symptoms of surviving plants at the periphery of "lightning spots." These symptoms have been interesting not only because of the nature of plant response, but because of their diagnostic value. As the electric charge reaches the earth, plants in the immediate vicinity are killed; they wilt and desiccate rapidly. The charge moving radially along the surface of the soil is eventually dissipated to such a point that the plants encountered are injured, but not killed. They are stunted in various degrees. A few weeks later the typical symptoms may be found.

Probably more often than not the upper layer of soil has become moistened slightly before the lightning strikes. When moist, it aids in dispersion of the current radially. The injury to surviving plants is at the ground level on the side of the stem facing the center of the damaged area. A relatively small surface lesion is formed, for the discharge passes through cortex, phloem, and xylem without producing appreciable injury in those tissues. Having reached the pith, which in half-grown plants is still solid but made up of thin-walled parenchyma cells, the charge progresses through the tissue until spent. The result is the death of the pith cells, their collapse, and the formation of a hollow region surrounded by a dark brown

to black layer of collapsed, desiccated tissue. Orton has mentioned the destruction of pith in cabbage injured by lightning.<sup>1</sup> Adventitious roots are commonly formed within this cavity and specimens have been collected in which this abnormal root development had proceeded until the cavity was completely filled. In plants that survive, the cavity, ordinarily, does not extend into the stele of hypocotyl and root. A typical case of a pith cavity is shown in figure 1, B and C.

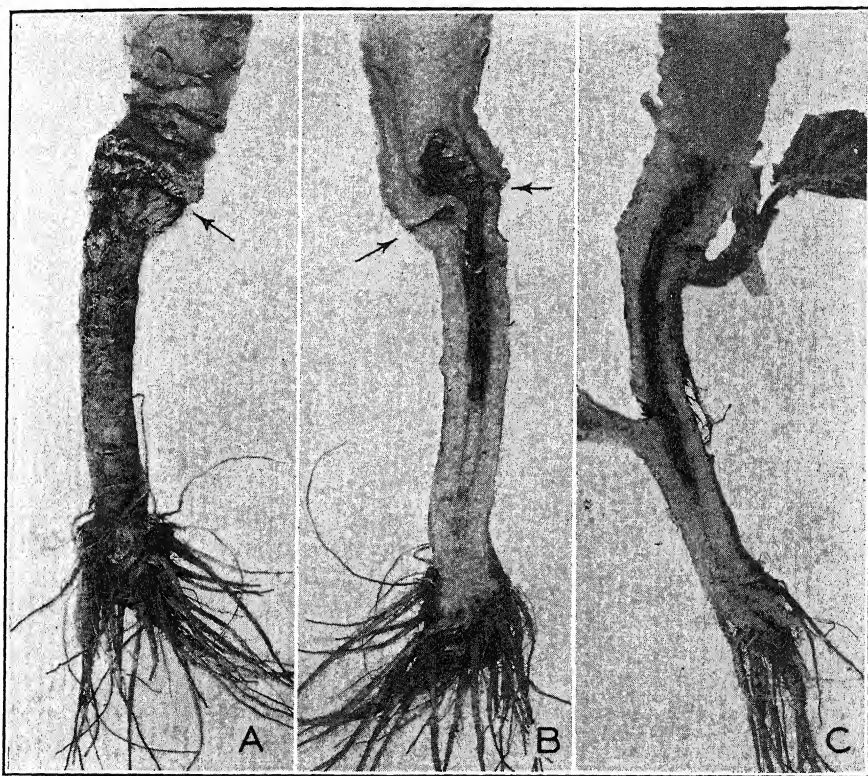


FIG. 1. Lightning injury to cabbage. A. External view of plant several weeks after injury. The arrow points to scar overgrown by callous tissue. B. Longitudinal section of the same plant shown in A. The arrows point to entrance or exit of electric current through the cortex and the vascular tissue. The characteristic pith cavity contains many adventitious roots. C. An injured plant in which dormant buds were stimulated at the leaf scars immediately below the points at which the electric current penetrated the stem.

The injury to the cortex and the vascular ring often is reduced to a narrow line in cross section. (Fig. 1, B.) The external appearance of the same plant is shown in figure 1, A. The point of initial injury has become

<sup>1</sup>Orton, C. R. Lightning injury to potato and cabbage. *Phytopathology* 11: 96-98. 1921.



covered with callous tissue. There is only slight damage to the cortex below the callous region. A common response of injured survivors is the stimulation of the bud at the leaf scar next below the point of cortical injury. In the plant illustrated in figure 1, C, the cortex was injured at two points, and the buds elongated below each point of entrance.

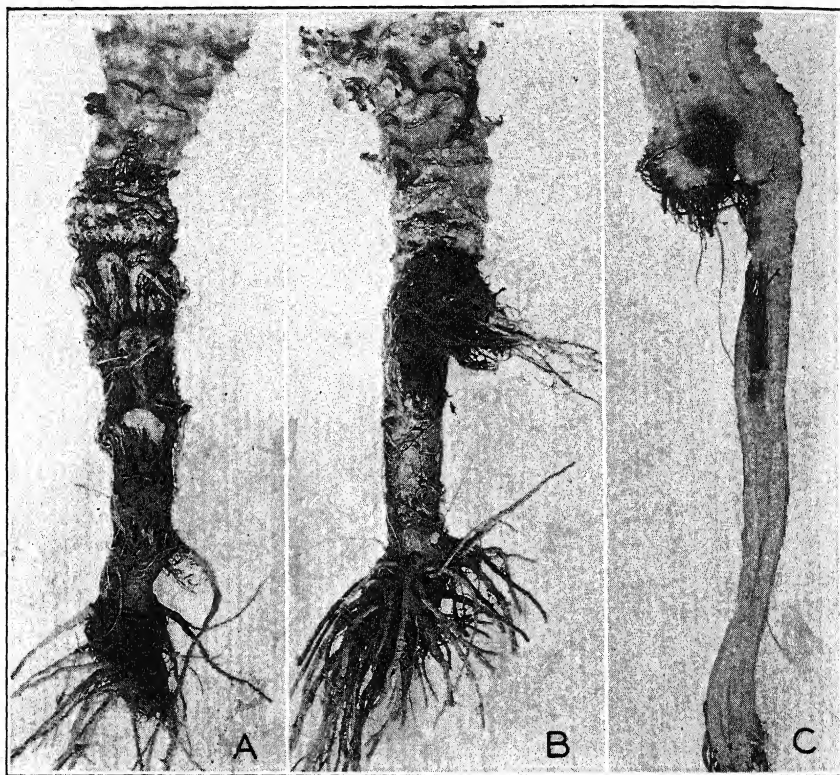


FIG. 2. Lightning injury to cabbage. A. Marked injury to the cortex of the subterranean stem, which was followed by unusual formation of adventitious roots at the leaf scars. B. Severe stem injury with the formation of adventitious roots above the lesion. C. A plant in which severe injury at the soil line was followed by the development of adventitious roots. The latter supplemented the support of the normal roots with the result that the stem above the lesion enlarged more rapidly than that below it.

When the surface damage is more severe, adventitious roots are stimulated above the point of injury (Fig. 2, B). If the charge damages the subterranean cortex generally, adventitious roots may arise from all leaf scars involved (Fig. 2, A). In figure 2, C, a seriously damaged survivor is shown in longitudinal section. The injury at the soil line was followed by excessive root formation and concomitant stunting of the lower stem. Nearly



normal enlargement of the upper stem proceeded, however, from the combined support of the normal and adventitious root systems.

If the damage is chiefly to the pith, the shock to injured survivors is temporary and they may recover rapidly and head normally. If the surface damage is severe (Fig. 2, A and C), the plant's growth is reduced and its yield is diminished accordingly.—J. C. WALKER, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, and Department of Plant Pathology, University of Wisconsin.

*Maternal Inheritance in Pears.*<sup>1,2</sup>—The breeding of pears has to recommend it not only the usual considerations, such as increased purity of parent clones and improvement in horticultural types, but also the urgent need for resistance to fire blight (caused by *Erwinia amylovora*), which has been instrumental in limiting the culture of the better varieties of pears over large agricultural areas. Little is known specifically of the inheritance in pears, due no doubt to the extreme variability among individuals grown from the seed of a species or of a single horticultural variety. Kikuchi<sup>3</sup> reports russet skin dominant to smooth skin in *Pyrus serotina*, while Wellington<sup>4</sup> states that in *P. communis* the smooth green skin is dominant to russet brown skin. Tufts<sup>5</sup> has noted that among trees from seed of the hybrid variety LeConte the individuals with persistent and deciduous calyces have a definite ratio (3 to 1 or 1 to 3) to each others.

In some observations on trees from reciprocal crosses of cultivated varieties of pears a seeming tendency was noted toward a closer resemblance of the F<sub>1</sub> individuals to the seed parent than to the pollen parent. The present preliminary report is limited to such observations on crosses of several horticultural varieties of *P. communis* with the variety Kieffer, which is presumed to be a hybrid between *P. communis* and *P. serotina*.

Of the 9 tree characters studied, 3 that are typical of the Kieffer parent are selected as sufficiently tangible to be used as evidence (Table 1). It is evident that some influence resident in the maternal parent is operating for these characters.

*Resistance to Fire Blight.* It was of interest then to determine whether the reciprocal hybrids reacted in the same way with reference to blight resis-

<sup>1</sup> The authors wish to express their appreciation to Professor R. Wellington who furnished the materials for this study.

<sup>2</sup> An abstract of this work has already been published: Hildebrand, E. M., and S. L. Hsiung. Inheritance of plant characters and resistance to fire blight in pear. (Abstract) Phytopath. 27: 131. 1937.

<sup>3</sup> Kikuchi, A. On the skin color of the Japanese pear and its inheritance. (In Japanese.) Cont. Inst. Plant Industry Kyoto Imp. Univer. 8: 1-50. 1930.

<sup>4</sup> Wellington, R. Inheritance of the russet skin in the pear. Science (n.s.) 37: 156. 1913.

<sup>5</sup> Tufts, W. P. An inquiry into the nature of a somatic segregation of characters in the Le Conte pear. Oregon Agr. Exp. Sta. Bull. 123. 1914.

TABLE 1.—*Inheritance of tree characters, and the results of infection in reciprocal crosses of pear varieties artificially inoculated with Erwinia amylovora*

Varieties crossed	Seedlings	Seedlings with large lanceolate leaves	Seedlings with sharply serrate leaves	Large lenticels on branches	Shoots inoculated	Shoots infected	Av. length of lesion
	Number	Per cent	Per cent	Per cent	Number	Per cent	Inches
Kieffer × Phelps .....	37	84.6	80.8	81.0	120	12.5	3.24
Phelps × Kieffer .....	14	25.6	37.5	29.4	80	55.0	5.36
Kieffer × Flemish Beauty .....	32	68.2	84.0	72.4	145	15.1	2.64
Flemish Beauty × Kieffer .....	7	33.3	40.0	45.6	40	45.0	4.42
Kieffer × Pulteney...	30	52.4	61.9	.....	107	26.2	3.40
Pulteney × Kieffer...	21	16.6	42.8	.....	130	49.2	5.08
Kieffer × Seckel .....	116	83.5	a	.....	301	27.7	3.22
Seckel × Kieffer .....	14	10.0	a	.....	98	31.6	2.35

a This character is similar for both parents.

tance as with external tree characters. The percentage of shoots infected and length of shoot killed, following inoculation at the tip, was selected as a measure of the degree of resistance, since the blossoms of all the varieties seemed about equally susceptible and since it was not feasible to make inoculations in the trunks. As a basis for comparison, inoculations were made in from 36 to 77 shoots of each of the parent varieties. The average length of blighted parts (in inches) was about equal for Kieffer (2.37) and Seckel (2.82) and distinctly greater for Flemish Beauty (5.90), Phelps (5.64) and Pulteney (5.73). The respective percentages of shoots infected for these varieties were 35, 40, 70, 76, and 71. When inoculations were made on trees of the  $F_2$  generation of reciprocal crosses the tendency toward maternal inheritance was again noted (Table 1). Since both parents of the crosses between Kieffer and Seckel are about equally susceptible, it is rather to be expected that the progenies of these crosses will be similar in degree of resistance. In all other crosses treated in table 1, those with Kieffer as the seed parent were significantly less susceptible than the corresponding reciprocal crosses.

These results are believed to indicate the existence of true maternal inheritance in the pear. A similar phenomenon has been reported by Parker<sup>6</sup> on the inheritance of resistance to common mosaic virus in the bean.—S. L. HSIONG and E. M. HILDEBRAND, Departments of Pomology and Plant Pathology, Cornell University, Ithaca, New York.

<sup>6</sup> Parker, M. C. Inheritance of resistance to the common mosaic virus in the bean. Jour. Agr. Res. [U.S.] 52: 895-915. 1936.

*A Strain of Alternaria citri Ellis and Pierce causing a Leaf Spot of Rough Lemon in Florida.*—Doidge<sup>1</sup> has shown that a fungus similar to *Alternaria citri* causes a leaf spot of the rough lemon, *Citrus limonia* Osbeck, in South Africa, and states that this spot is widely distributed in citrus nurseries, especially in the more humid districts. On this host in Florida, a species of *Alternaria* has been observed frequently in leaf spots associated with the withertip fungus, *Colletotrichum gloeosporioides* Penz., but generally has been considered to be secondary to the latter.<sup>2</sup>

In 1936 and 1937 a leaf spot similar to the one described by Doidge was abundant and caused considerable defoliation on rough-lemon seedlings in several nurseries in Dade County, Florida. In the majority of the spots examined a species of *Alternaria* was fruiting in the dead areas without evidence of the presence of another fungus. In many of the spots *Colletotrichum gloeosporioides* was sporulating with the *Alternaria* and was typically the dominant fungus where the mixed infection occurred.

The spots vary in size, but usually do not exceed 2 cm. in diameter unless secondary infection by *Colletotrichum* occurs. They are subcircular at first, but become more or less irregular in shape because of the invasion advancing more rapidly along the veins of the leaf, and frequently they show distinct zonations (Fig. 1). The color ranges from light to dark brown, the margins typically darker than the centers. When multiple infections occur on a single leaf, the entire leaf blade becomes chlorotic. Such leaves show a tendency to curl upward and drop prematurely from the tree. When only 1 or 2 spots occur on a leaf, infection may persist on the tree and the dead areas weather away, leaving a jagged hole.

In older spots, spores are produced abundantly on tufts of conidiophores, appearing as small black specks scattered irregularly over the surface of the dead area. As found on the leaf, the muriform spores are obelavate and elongate, fuscous, 40 to 70  $\mu$  long and 12 to 20  $\mu$  broad at the rounded base, tapering towards the apex, which elongates into a narrow, subhyaline beak, ranging from 7 to 35  $\mu$  long. Transverse septa vary from 3 to 9, the usual number being 6 or 7, and longitudinal septa occur in all but 2 or 3 apical cells. The cells generally are more or less constricted at the septa.

The fungus grows readily and fruits rather abundantly on common laboratory media. The spores produced on media average somewhat smaller than those produced on leaves. A few were observed possessing beaks up to 11  $\mu$  long; but, generally, these structures were absent.

A culture of *Alternaria citri* Ellis and Pierce, isolated from a fruit rot, was obtained from the Florida Agricultural Experiment Station at Gaines-

<sup>1</sup> Doidge, Ethel M. A study of some *Alternarias* affecting citrus in South Africa. Union So. Africa Dept. Agr. Sci. Bull. 69. 1929.

<sup>2</sup> Fawcett, H. S. Citrus diseases and their control. Ed. 2, 656 pp. McGraw Hill Book Co., New York and London. 1936.

ville for comparison with the leaf-spotting strain. When grown in parallel cultures on various media, the 2 forms were almost identical. Minor variations in color of the colonies and in abundance of sporulation were observed, but there were no distinct differences that could be regarded of diagnostic value. The form isolated from leaf spots is morphologically similar to *A. citri* and is considered a strain of that species.

Leaves of potted seedlings of the rough lemon and the Rangpur lime were inoculated under bell jars by placing drops of a spore suspension on both

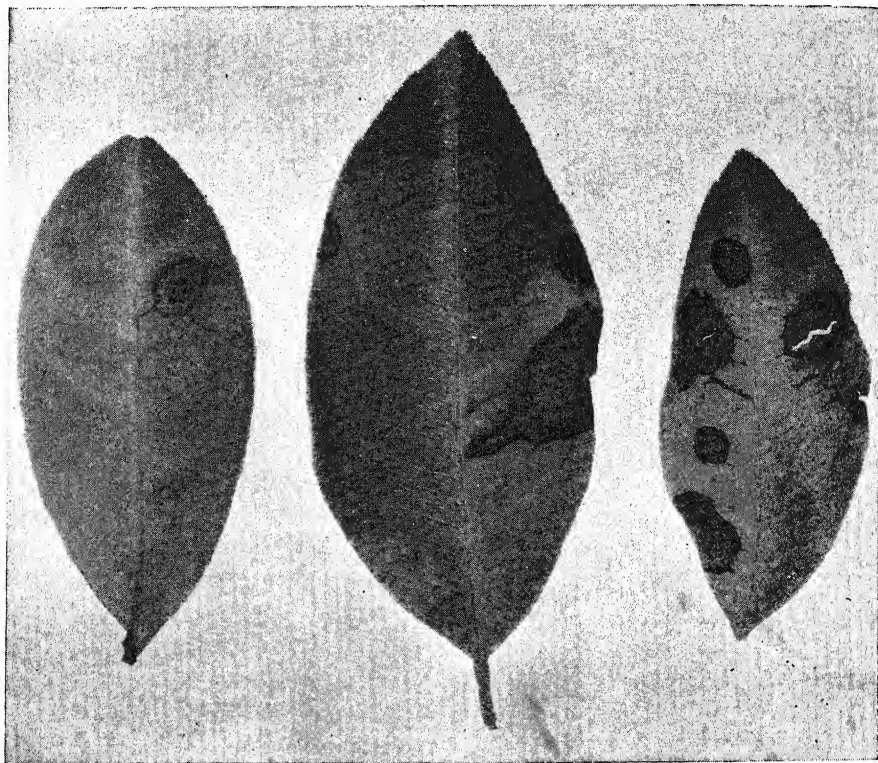


FIG. 1. Spots produced by *Alternaria citri* on leaves of rough lemon.

young and mature leaves. The spore suspension was made with sterile water from single spore cultures of the leaf-spotting *Alternaria*. In 4 days, infections were plainly visible on the young leaves as small, pale green areas with brownish centers, and, 4 weeks after inoculation, had developed into typical spots with spores of the fungus present. Cultures made from these spores were identical with the original cultures. The inoculated mature leaves and leaves of control plants did not develop typical leaf spots. Inoculations made with spores of the fruit-rotting strain also gave negative results.

The leaf-spotting strain produced a typical *Alternaria* rot when inoculated into surface-sterilized oranges and lemons, but did not sporulate in the decayed tissues. The fruit-rotting strain produced a more rapid decay of lemons and produced spores in the fruit tissues.

While adequate control measures have not been worked out, the application of Bordeaux mixture, for the control of citrus scab, reduces the severity of *Alternaria* infection.—GEO. D. RUEHLE, Sub-Tropical Experiment Station, Homestead, Florida.

*The Variable Properties of Potato as a Bacteriological Culture Medium.*—Plant pathologists and mycologists who use potato tissue or potato extract for the cultivation of fungi or bacteria will be interested in the abstract of a paper by Janet McCarter and E. L. Tatum appearing under the foregoing title in the *Journal of Bacteriology*, Vol. 33, pp. 30 and 31, Jan., 1937. The abstract serves to emphasize the necessarily variable composition of media made from plant or animal material and illustrates the difficulty of using such media to provide facts on rate and character of growth capable of satisfactory comparison with those obtained by others or by the same workers at different times.—H. P. BARSS, Office of Experiment Stations, U. S. Department of Agriculture, Washington, D. C.

*Observations on the Yam Nematode (Rotylenchus bradys (Steiner & LeHew, 1933)) Filipjev, 1936.*—It is proposed to designate the tylenchid nematode, *Rotylenchus bradys*, occurring parasitically on yams (*Dioscorea*), with the vernacular name yam nematode. The form is at present recorded from only this particular host. T. Goodey,<sup>1</sup> on authority of J. West, mentions the species *Dioscorea alata* Linn., *D. cayenensis* Lam., and *D. rotundata* Poir. as being attacked in Nigeria, West Africa. Various yam tubers infested by this nematode submitted to the writer by N. R. Hunt, Bureau of Entomology and Plant Quarantine, from Jamaica and Puerto Rico unfortunately could not be identified as to species, except in the case of a recent finding from Cuba. The latter, according to R. A. Young, Bureau of Plant Industry, U. S. Dept. of Agriculture, probably was *D. alata*. Here the yam tuber was still in relatively good condition and, for the first time, we could closely observe the disease symptoms exhibited by the tuber. The features are those of a dry rot. The surface of the yam (Fig. 1, A) is rough, with cracks exposing the subdermal, at this stage almost black, layers. Cross and longitudinal sections (Fig. 1, B, C, D, E, F) show this black layer under the entire surface forming irregular protuberances inward. Here and there isolated infection centers appear nearer the center of the tuber. Their color

<sup>1</sup> Goodey, T. Observations on a nematode disease of yams. *Jour. Helminthol.* 13: 173-190. 1935.

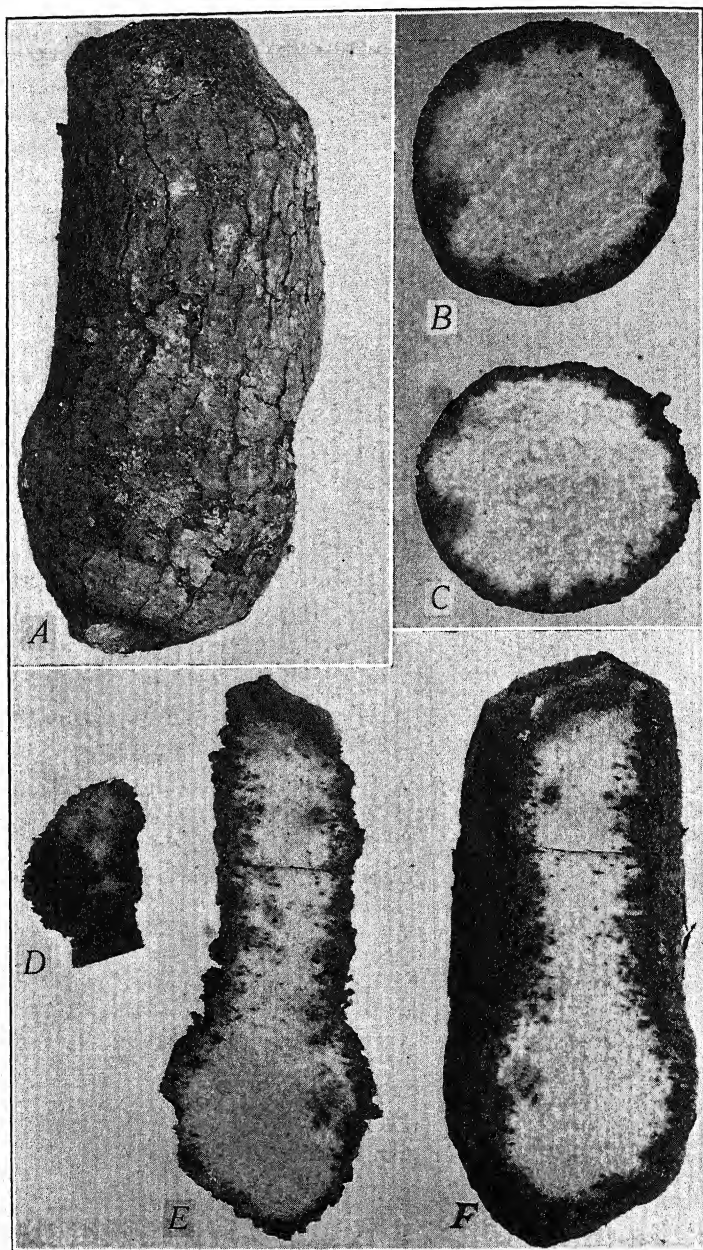


FIG. 1. Yam tuber attacked by the yam nematode, *Rotylenchus bradys*. A. Surface view. B and C. Cross sections through the tuber exhibiting the blackish infested region under the skin. D-F. Tangential longitudinal sections through tuber; infection appearing also in spots.  $\times \frac{1}{2}$ .



is first a pale yellow, turning to deep brown and blackish as the infestation progresses.—G. STEINER, Bureau of Plant Industry, U. S. Dept. of Agriculture, Washington, D. C.

*Abnormal Germination Resulting From Improperly Galvanized Trays.*<sup>1</sup>—In December, 1935, several lots of solanaceous seed germinated erratically on blotters and the tests were repeated in soil. Since the latter trials indicated seed of normal viability it seemed evident that the galvanized trays used for holding the blotters were supplying a phytocidal property.

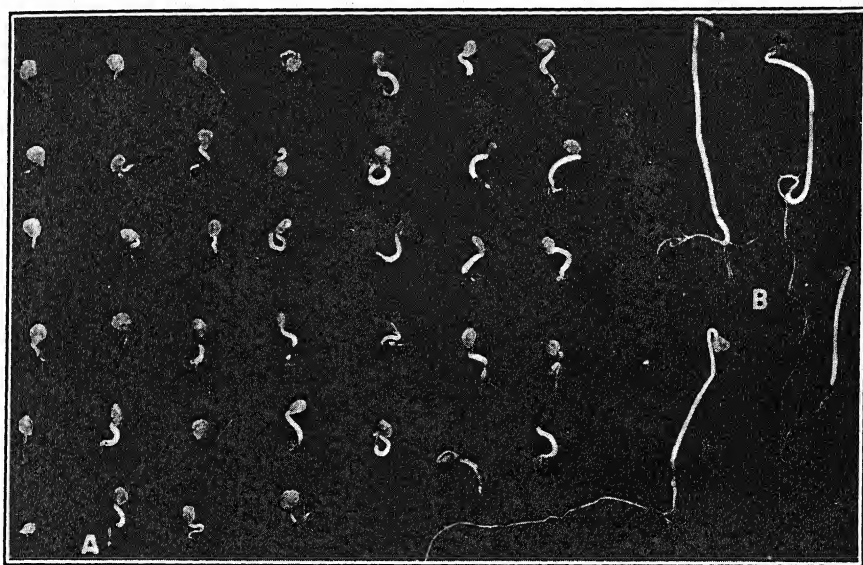


FIG. 1. Tomato seedlings photographed 12 days after seeds were placed to germinate on blotters. A. Severely stunted seedlings showing inhibition or killing of radicles. B. Nearly normal seedlings taken from zinc-free spots of the same blotters.

The radicles of the developing seedlings varied from  $\frac{1}{2}$  to 4 inches in length at the end of the ten-day germination period. Tomato seedlings (Fig. 1) were distinctly injured. The shorter roots were discolored, curled, roughened, and usually markedly shrivelled at the tips. The plumules, when they developed, were normal in color and orientation but exhibited slight nonuniformity in length. Similar injuries had been observed previously in tests of seeds treated with zinc oxide, so suspicion was directed toward a soluble zinc salt in the condensation water, as mentioned by Kadow *et al.*<sup>2</sup> Moreover, the

<sup>1</sup> Approved by The Director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 196.

<sup>2</sup> Kadow, K. J., W. A. Ruth and H. W. Anderson. Greenhouse wires and pipes galvanized with zinc react with sulphur dioxide to form soluble zinc salts. *Phytopath.* 26: 609-610. 1936.

aberrant seedlings often were confined to circular spots surrounded by apparently normal sprouts. Meshes of the hardware cloth in the trays immediately above contained chalky white drops of water. Frequently, seedlings on these trays also were injured, so that even approximate germination readings were impossible. Irregular grayish white streaks in the blotters coincided with the areas of abnormal radicles.

Qualitative chemical analyses demonstrated zinc in both the drops of water and the discolored portions of the blotters. It seems probable that the metallic zinc used to galvanize the hardware cloth was converted into either zinc oxide or zinc carbonate.

When the trays were placed in a humid germinator the zinc compound passed into solution in water and was deposited by sorption in the blotters both on the defective trays and, through drip water, on the trays immediately below.

Dipping the trays in water caused no diminution in the severity of the injury and neither was all of the soluble zinc removed by a prolonged, weak-nitric acid soak. In January of 1937 several blotters of flower and vegetable seeds were used to determine if the toxic property had decreased in storage. Characteristic small, deformed radicles developed in percentages equal to those in tests conducted 13 months before.—W. F. CROSIER, S. R. PATRICK, and M. T. MUNN, N. Y. State Agricultural Experiment Station, Geneva, N. Y.

*An Indication of Seed Transmission of Mosaic Virus in Tomato Seed.*<sup>1</sup>—During the fall of 1935, tomato plants grown from seed saved from a selection of the variety Indiana Canner at Medford, Oregon, yielded some plants affected with mosaic. The seed had been planted November 1, 30 days after the seed had been removed from the fruit. Although the usual precautions were taken to prevent accidental mosaic infection since the seedlings were intended for tomato-virus studies, 4 of the 25 transplanted plants developed mosaic symptoms. At the same time another lot of seeds of the same variety, but from another source, was planted under similar conditions; these developed seedlings entirely free from mosaic. Another series of plants was grown from the same seed source 2 months later, and again 3 of the 25 transplanted plants developed mosaic symptoms, while the 25 control plants treated in like manner, remained disease-free. When the sources of the seed were compared, it was found that the lot of seed that developed the plants showing mosaic symptoms, had been saved from plots where mosaic had occurred in nearly all of the plants. As no special care was taken to prevent the spread of any masked virus that might have been present when the plants were trans-

<sup>1</sup> Published as technical paper No. 257, with the approval of the Director of the Agricultural Experiment Station, contribution of the Botany Department.

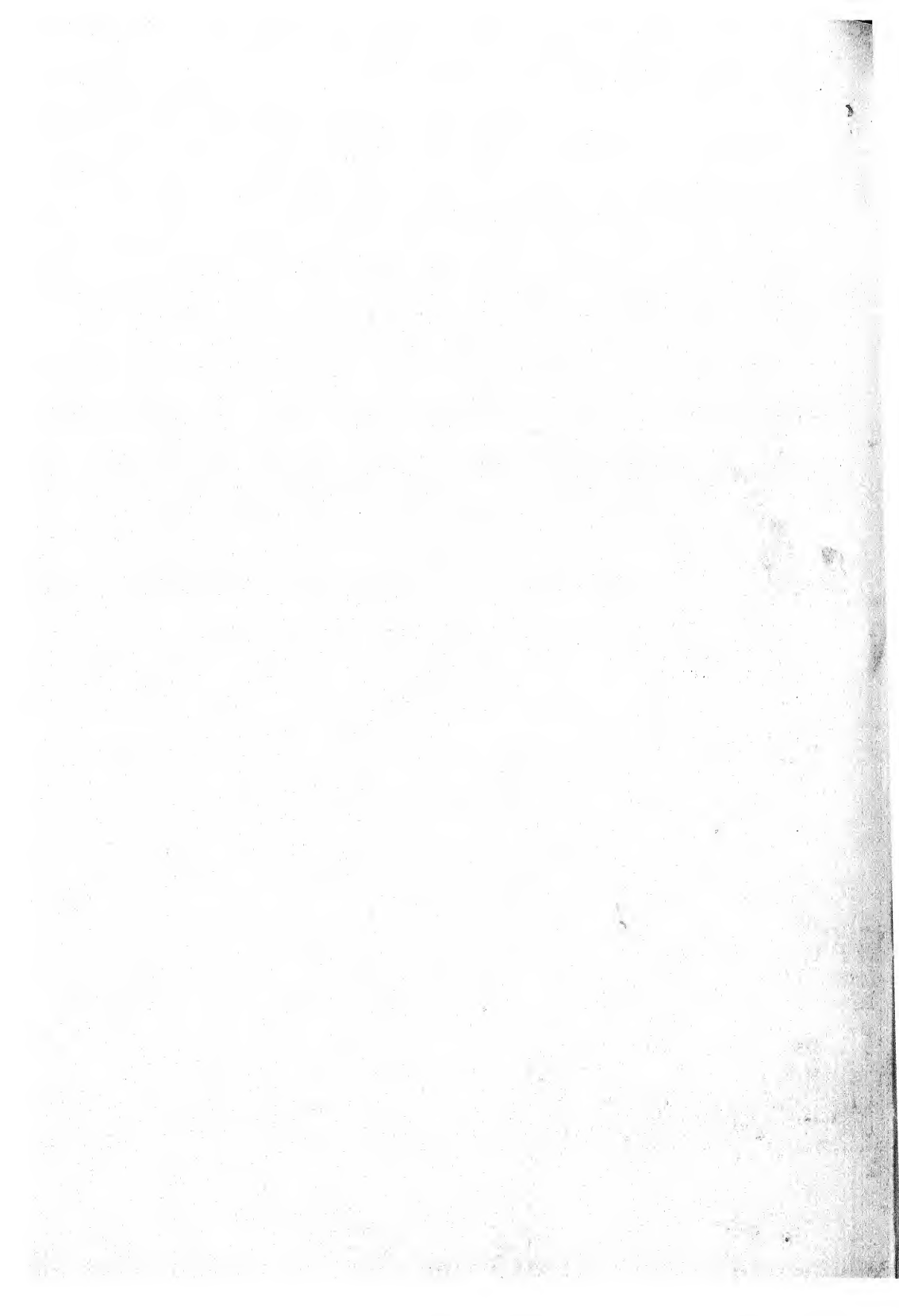


planted, the above proportions of mosaic plants probably are too high, but the cases do indicate that some mosaic was seed-borne.

Six months later another sample of these seeds was planted in flats and placed in an isolated room, where extreme precautions against possible mosaic contamination were taken. The plants were not handled in any manner until they had reached the 3- to 4-leaf stage; then the number of mosaic plants was determined. Five plants out of 677 showed typical mosaic symptoms.

At the end of the 1936 season, seed was collected again from field plants of Indiana Canner showing mosaic symptoms. Three weeks later this seed was planted in 5-inch flower pots, which were carefully isolated to prevent contamination. These plants were not handled in any manner until they had reached the 3- to 4-leaf stage, when they were examined for mosaic. Eleven plants out of 168 grown in this manner were affected with mosaic.

It may be significant that seeds aged 3 weeks produced 11 mosaic plants out of 168, while those aged 9 months produced only 5 mosaic plants out of 677. It appears that the tendency to transmit the virus depends on the age of the seed. In the course of our studies of the tomato tip-blight virus, we have handled thousands of tomato plants, but only this selection of Indiana Canner evidenced mosaic in a manner suggestive of seed transmission. Since so much conflicting evidence has been published on this subject, the above observations are deemed of interest.—J. A. MILBRATH, Oregon State College, Corvallis, Oregon.



# SOME PHYSICAL, CHEMICAL, AND BIOLOGICAL PROPERTIES OF A SPECIFIC BACTERIOPHAGE OF PSEUDO- MONAS TUMEFACIENS<sup>1</sup>

G. C. KENT<sup>2</sup>

(Accepted for publication March 12, 1937)

Abundant evidence has been obtained to demonstrate that bacteriophages are associated with numerous plant and animal pathogens. However, the specific properties and rôle of these phages still remain a challenge to plant and animal pathologists.

The study of the properties of the phages of bacterial plant pathogens has progressed slowly because of the greater emphasis placed on other biological studies relating more directly to the economic phases of bacterial diseases. Phages have been shown to be present in a number of plant organs and in soil and water with which diseased plant materials have been mixed. In only a few of the phages studied have the titre, thermal inactivation, range of susceptibility, and similar properties been determined. Furthermore, little attempt has been made to use standardized procedures: For example, the time of thermal inactivation tests, when stated at all, varied from 10 to 30 minutes; the titre was based on plaque or dilution methods; and there was seldom any indication of the use of only one phage isolate in the determination of the characters. In many cases it was assumed that all phage isolates active on one species of organism were the same. These inconsistencies, coupled with the incompleteness of the available data, seriously interfere with the identification and determination of the properties of a new isolate.

A complete understanding of the action and uses of phage isolates will be possible only when new strains may be identified on the basis of previous work. In order to identify phage strains it will be necessary to determine the properties and biological activities of the lytic principles under standardized procedures. The phages show evidence of as wide a range of "parasitic" relations as do bacteria or fungi, and, in order that the necessary classification of the different phages may be constructed, a number of separate ones must be thoroughly investigated and the characters capable of use in such work discovered. With this thought in mind, the following investi-

<sup>1</sup> Taken from a thesis submitted to the faculty of the Graduate College, Iowa State College, in partial fulfillment of the requirements for the degree, doctor of philosophy. Project No. 478 of the Iowa Agricultural Experiment Station, Ames, Iowa. Journal Paper No. J 403 of the Iowa Agricultural Experiment Station. One-half the cost of publication of this paper was borne by the Iowa Agricultural Experiment Station.

<sup>2</sup> The author wishes to express his sincere appreciation to Dr. I. E. Melhus for suggesting the problem, for his stimulative counsel during the study and his encouragement during the preparation of the manuscript.

gation was undertaken to determine certain of the physical and biological properties of a single uniform strain of phage active on a well-known plant pathogen, namely, *Pseudomonas tumefaciens* Sm. and Town.

#### PERTINENT LITERATURE

The literature dealing with the bacteriophages of animal pathogens has been thoroughly reviewed by Bronfenbrenner (5, 6), Burnet (11, 12), Hadley (28), and others, and its repetition here would be superfluous. It does seem essential, however, to assemble the literature concerning the phages of plant pathogens.

The association of a bacteriophage with a plant pathogen was first studied by Gerretsen, Gryns [Grÿns], Sack, and Söhngen (26) in 1923. Gerretsen and his coworkers obtained the phage from the roots and stems of a number of nodule-bearing legumes, but failed to find it in the leaves. They could recover the lytic principle from garden and field soil, but not the soil of woods and heaths. The phages were found to diffuse through colloidal and to resist drying on filter paper for 2 months. The serradella strain was inactivated at a temperature of 60° C. in 30 minutes, while the clover and lupine strains resisted a temperature of 65° C. for 15 minutes. These authors reported that the phages were specific for the bacterial strain isolated from the species of plant in the locality from which the lytic principles were obtained. Although Gerretsen *et al.* could not obtain plaque formation, Grijns (27) later obtained it and reported that the phage was not produced in the roots of plants grown in pure culture with nonlysogenic strains of bacteria. Absence of phage did not prevent development of nodules.

Demolon and Dunez (19), Hitchner (29), Laird (33), and Vandecaveye and Katznelson (54) have verified the isolation of the phage from roots, stems, and cultivated soils. Hitchner (29), after considerable difficulty, obtained a phage that cleared the bacterial culture slowly and had a titre of only  $1 \times 10^{-4}$  to  $1 \times 10^{-5}$  after 20 passages. He found that numerous resistant secondary cultures were formed that would lose their resistance after 6 months within the host plant. Under similar passage through the host, the susceptible cultures retained their original nature. Hitchner found the same strict specificity reported by Gerretsen *et al.*

Demolon and Dunez (19), also, failed to find the phage in leaves or in noduleless plants, while they obtained it over 30 cm. from the noduled roots in the soil. They stated that the phage sterilized the soil, so far as the legume bacilli were concerned, and attributed "running-out" of alfalfa to this fact. These authors isolated, also, the phages from stock cultures. Their tests of the phage showed a low titre,  $1 \times 10^{-4}$  to  $1 \times 10^{-7}$ , which may account for the slow clearing, 48 to 72 hours. The tests of specificity indicated a

wide variation in susceptibility of the bacteria and virulence of the phage strains.

Laird (33) obtained phages from stock cultures of *Rhizobium trifolii*, as well as the plant nodules. The phages were transmissible from liquid or plaque culture and showed a titre of  $1 \times 10^{-8}$ . They were not specific for the strains of *Rhizobium* with which they were isolated, and a marked variation in susceptibility was shown by the progeny of single-cell isolates. In general, he found that the susceptible strains were more vigorous in the production of nodules than the resistant strains.

Izrail'skiĭ (31) found a lack of strain specificity in phages associated with the legume bacilli. When tested on *Bacillus radicola*, *B. radiobacter* and *Pseudomonas tumefaciens*, he states that the phages specific for each species could only be used as confirmative evidence for determination and then only when positive evidence was obtained.

Vandecaveye and Katznelson (54) obtained phages from stock cultures and from soil that had been in alfalfa for over 2 years. Their phage showed a high titre,  $1 \times 10^{-11}$ , and was said to be responsible for the poor nodulation of plants in the soil harboring it.

One of the first phages of a plant pathogen isolated was taken from a rotted cabbage head by Mallmann and Hemstreet (39), in 1924. However, the bacterium involved was not determined. It was shown that the phage was inactivated in 30 minutes at 68° C., withstood a dilution of  $1 \times 10^{-12}$ , and, although active at first on several organisms, it soon lost this capacity.

Anderson (1) reported the isolation of a phage for *Pseudomonas pruni* from the soil under an infected peach tree, but could not isolate the phage from diseased leaves. Filtration studies of this phage by Thornberry (52) showed it to have an approximate diameter of 11  $\mu$ .

Bewley (3) isolated a phage active on a grey bacterium taken from a tomato stem. He suggested that this phage might enter the tomato on the bacteria, become adapted to the host, and produce the aucuba mosaic.

Uppal (53) reports the isolation of a phage specifically associated with *Pseudomonas citri*, but has not investigated it further.

Commencing in 1931, Massey<sup>3</sup> (41) has consistently isolated phages for *Pseudomonas malvacearum* from fallow or cultivated land that bore an infected cotton crop, and from flood waters of the Blue Nile, although neither clean land nor the clear waters of that river have yielded the phage. He was able, by repeated inoculations with a nonlysogenic strain of *Ps. malvacearum* to produce the phage in garden soil.

A phage specific to *Pseudomonas solanacearum* in its action was isolated

<sup>3</sup> Massey, R. E. Report[s] on experimental work. Sudan Govt., Gezira Agr. Res. Serv., Ann. Rept. 1933: 126-146. 1934; 1934: 119-141. 1935. [Mimeographed.] [Abstracts in Rev. Appl. Mycol. 13: 696-697. 1934; 14: 756-757. 1935.]

from infected tomato tissues by Matsumoto and Okabe (42). It was found to be inactivated at 65° C. in 3 minutes, and antigenic in nature.

Thomas (51) found that a phage for *Bacterium stewartii*, obtained from heavily decayed roots of infected sweet corn, was capable of reducing the infection from 18 per cent in plants from nontreated seed to 1.4 per cent in plants in which the seed had been soaked for 48 hours in the phage filtrate. Leaf inoculations with phage plus bacteria showed a similar reduction in infection. Although the titre of the phage was  $10^{-7}$ , the clearing of the culture was never complete.

Moore (43) in South Africa first reported a phage specifically associated with *Pseudomonas tabacum*, which was obtained from the juice of diseased leaves. Although the principle did not produce complete lysis, it was transmissible and resisted considerable heating and storage. The lysis was demonstrable only in bouillon or saline solution. Dufrénoy (24) studied the action of a phage on *Ps. tabacum* by staining the phage-supporting cells with neutral red. He found the swelling traceable to an increase in the vacuolar system. Other cells, not swollen, possessed small vacuoles, which absorbed the stain, and larger vacuoles in which the precipitated sap did not absorb the dye. Cells that failed to swell or take up the dye usually were found agglutinated by the phage. Dufrénoy considered these characters as evidence for the grouping of this phage with the viruses of plants that show a similar effect on their hosts.

The existence of polyvalent phages associated with plant pathogens has been reported by two sets of workers. Brown and Quirk (10) reported the presence of virulent phages, active on *Bacillus carotovorus* and *Pseudomonas tumefaciens*, in filtrates obtained from artificially induced galls and from vegetables showing soft rot. Weaker phages were present in filtrates of healthy carrots. Diluted phage inocula increased the growth and pathogenicity of the associated bacteria, while no evidence was found of the formation by phage exposure of filterable bacterial forms capable of the production of galls, as suggested by d'Herelle and Peyre (23).

Coons and Kotila (18) obtained polyvalent phages from rotted carrot, soil, and river water that caused definite lysis of *Bacillus carotovorus*, *B. atrosepticus* and *Pseudomonas tumefaciens*, inhibited the growth of *B. amylovorus* and *B. typhosus*, but were inactive on a number of other organisms tried. The phage, which was found to vary greatly in its toxic action from day to day and with extent of seeding and inocula, maintained an average titre of  $10^{-8}$ . Lysis was produced by the phage over a temperature range of 7.8° C. to 36.1° C., with a definite maximum at 25° C. A reduced activity of the phage was exhibited after 5½ months' storage in sealed flasks. The phage caused a malformation and agglutination of the bacteria preceding the actual clearing, and on being spread in a thin film over susceptible vegetables, prevented the destructive development of the soft-rot organisms.

Phages associated with *Pseudomonas tumefaciens* have been the object of considerable investigation by a number of workers. Izrail'skiĭ (30, 31) obtained a phage of *Ps. tumefaciens* from a galled beet. The phage was active at all temperatures allowing bacterial growth, was inactivated at 70° C., and showed a titre of  $1 \times 10^{11}$ . Izrail'skiĭ reports the same clumping and precipitation of the organism preceding clearing as reported above by Coons and Kotila. Of the 9 strains of bacteria tested, only 3 were susceptible, and from clouded areas of these, resistant secondary cultures were isolated. The phage was said to be of value as a means of determining *Ps. tumefaciens* only if used as confirmative evidence when a positive result was obtained.

Phages active on *Pseudomonas tumefaciens* were also obtained from sugar beet galls, virulent broth cultures, and sterilized and nonsterilized soil containing nonvirulent cultures of the organism by Muncie and Patel (44). They found the phage to be inactivated at 85° C. in ten minutes, inhibited by bile agar, and specific for the single strain of organism from which the lytic principle was isolated. The phage withstood a dilution of  $1 \times 10^{21}$  using a dilution technique which the authors stated might be somewhat questionable. A rough type of secondary culture gave a susceptible smooth form after exposure to phage action. No evidence was obtained of formation of resistant colonies.

Verona (55) failed to confirm the isolation of phages for *Bacillus carotovorus*, *B. radicola*, *Pseudomonas tumefaciens*, *Ps. pruni*, *Ps. campestre*, *Ps. hyacinthi*, *Ps. mori* and a number of saprophytes from the soil.

The necessity of adopting rigidly controlled methods for phage work was pointed out by Chester (16) in development of methods for the isolation of phages associated with *Pseudomonas tumefaciens*. Testing the various conditions that must be controlled, he stressed uniformity of bacterial inoculum, media, environmental conditions, and phage inoculum. In the experiments he found the phage present in galled pelargonium stems, and beet roots, in healthy tissue closely surrounding the galls, and in a small percentage of healthy beets grown in contaminated soil.

#### METHODS

The culture of *Pseudomonas tumefaciens* used in this work was an apple strain reisolated after 3 passages through tomato. In the phage tests, a 48-hour agar culture was suspended in the culture solution, diluted to 300,000,000 bacteria per ml., and 0.3 ml. or approximately 100,000,000 bacteria were used to seed 10 ml. of cultural solution.

The bacterial cultures were carried on a glycerinated beef-extract agar, containing 3 g. Difco beef extract, 5 g. Bacto peptone, 20 g. Bacto agar, and 50 ml. of glycerine per liter of medium. The culture solution employed for

the phage tests was a modification of that found by Chester (16) to be most successful, and was referred to as Chester's bouillon. The medium contained 2.5 g. Bacto peptone, 2.5 g. of C. P. sodium chloride, and 1.5 g. of Difco beef extract per liter of distilled water.

The phage was isolated by thoroughly grinding an internal portion of a gall, aseptically removed, in 10 ml. of Chester's bouillon previously seeded with approximately 300,000,000 bacteria, incubating for 48 hours, and filtering through a Chamberland-Pasteur L3 filter, to obtain the filtrate for the identification tests. In the later passages, tubes of the earlier test were similarly filtered to obtain the inoculum.

The Chamberland-Pasteur L3 filters, which were used to obtain bacteria-free inocula, were set up, sterilized for 1 hour at 15 pounds' pressure, cooled, and employed with a slight suction to speed up the filtration.

Since, in the identification tests, a check was necessary which would eliminate the possibility of the clearing being due to bacterial by-products, a bacterial filtrate was obtained by filtering a culture previously inoculated with bacteria and bacterial filtrate in a manner similar to the phage tests. This inoculum was then comparable to the phage inoculum in everything except the phage.

Having obtained these materials, the liquid or bouillon identification test of the phage was conducted, using 6 series of tubes so set up as to include all possible checks and to thoroughly settle the question of presence or absence of a phage. These series were:

1. Medium + phage + bacteria—phage test;
2. Medium + bacteria—bacterial control;
3. Medium + phage—phage control;
4. Medium—medium control;
5. Medium + bacterial filtrate + bacteria—bacterial filtrate test;
6. Medium + bacterial filtrate—bacterial filtrate control.

Series 1 and 5 consisted of 4 tubes each, while the others had 2 tubes each. The medium was sterilized in 9 ml. quantities in large-size test tubes (1.5 × 15 cm.) to which were added, after cooling, the 0.3 ml. of bacterial seed, 0.5 ml. of phage, or 0.5 ml. of bacterial filtrate alone, or in the proper combinations for the above series.

A positive test for the phage was obtained when the tubes of series 3, 4, and 6 remained clear, series 1 remained clear or was nearly so, and 2 and 5 became cloudy in 24 to 48 hours. The strength of the phage action was considered stronger the earlier the lysis in series 1 developed and the longer it remained.

The readings for density of the turbidity were made by comparing the tubes at 24-hour intervals by means of nephelometer tubes, made up according to the McFarland method (37). In reading the tubes, care was taken not to shake them more than necessary, as it was found that shaking the phage tubes markedly increased the development of secondary turbidity.



The bacteria continued multiplying after seeding, turbidity increased, until 8 to 20 hours after inoculation. The first clearing may be followed by a secondary turbidity before the permanent clearing at 20 hours. The clearing was first evidenced by agglutination and slow dissolution, as reported for other phages of *Pseudomonas tumefaciens* (18, 30). Tests of bacteria made at this time by dilution plates showed the formation of arborescent, star-like, submerged colonies; streaks on agar plates showed a complete lysis, lack of bacterial growth, except in certain spots where secondary colonies occurred. Tests of these cultures failed to show any resistance to the action of the phage.

Another method was used for phage concentration determination, namely, agar plates spread with bacteria, dried for 2 hours, and then inoculated with the phage at proper dilution, are held and observed for the appearance of "worm-eaten" areas (plaques).

By this method the phage plaque first makes its appearance as a group of small white or opaque spots that begin to appear in the culture about 15 to 16 hours after inoculation. These spots usually are aggregated and hence appear as numerous phagic centers. The spots commence to clear in the center after 2 to 3 hours, and in 4 to 6 hours the entire central portion becomes a plaque from 2 to 6 mm. in diameter. The edge of this plaque holds the spotted or more advanced moth-eaten appearance until the culture is about 40 hours old, when enlargement stops and the edge becomes the characteristic smooth two-ring margin of the normal colony.

In conducting these tests, the phage, filtered at 48 hours from inoculation, when the culture had become clear, was diluted 1-9 with the cultural solution, Chester's bouillon, and subjected to the conditions of the test; diluted to remove the toxic action on the bacteria of the test materials; and the tubes of series 1, 2, 3, and 4 inoculated. These tubes were read at 24-hour intervals for at least 96 hours and the readings of all tubes condensed and reported as presence or absence of the phage after the test.

In certain cases it was necessary to determine the strength of the phage after the test. The phage inoculum was diluted by the usual bacteriological dilution method used in water analysis. Each dilution, in Chester's bouillon, was used to run series 1 and 3, testing the presence of the phage and purity of the phage inoculum. One set each of series 2 and 4 was run for the entire test. One nontreated phage test was always run to prove the presence of the phage in the material used in the test. This series was not reported, as no tests were recorded unless this series was positive throughout.

#### SOME PHYSICAL CHARACTERISTICS OF THE PHAGE

The reactions of any unknown agent to certain physical factors have long been used in the characterization of that agent. In attempting, there-

fore, to define the phage of *Pseudomonas tumefaciens* used in these tests, its reaction to certain physical factors has been studied.

*Temperature.* The conflicting reports on the optimum temperature for lysis of *Pseudomonas tumefaciens* by the phage associated with it, necessitated a study of the temperature relation of the phage. Coons and Kotila (18), using their polyvalent phage on *Ps. tumefaciens*, reported an optimum clearing at 25° C. Izrail'skiĭ (30), with a phage specific for the crown-gall organism, found no variation as long as bacterial growth was permitted.

Three tests were run using series 1, 2, 3, and 4 of the liquid-phage test at each 5° interval from 5° C. to 35° C. The readings were taken for 168 hours, but, since all readings gave the same indication, only the 72-hour readings were included in table 1. These readings indicated that the phage

TABLE 1.—*The effect of temperature of 5° intervals on the lysis of Pseudomonas tumefaciens by its phage*

No. of test	Inoculum	5° C.	10° C.	15° C.	20° C.	25° C.	30° C.	35° C.
1	Phage .....	—	—	—	+	+	±	+
	Bacteria .....	±	+	++	+++	+++	++	++
2	Phage .....	—	—	—	—	+	—	±
	Bacteria .....	—	+	++	++	+++	++	++
3	Phage .....	—	—	—	+	±	±	±
	Bacteria .....	—	—	++	++	+++	++	++

— = Culture tubes clear.

± = Clarity of culture tubes doubtful.

+ to +++ = Range of turbidity from 0.05 to 0.2 ml. of barium sulphate in McFarland nephelometer.

was active at all temperatures employed, which extended virtually to the limits of growth of the supporting organism. There was then no optimum temperature for lysis; the phage lysed the associated bacteria at all temperatures at which they were capable of growing, as found by Izrail'skiĭ. It is worthy of note that the clearing was much more thorough in time and amount at temperatures away from the optimum for growth of the associated organism. This was due probably to the fact that the phage acted on the bacteria present and killed them immediately and thus effected permanent clearing. At the optimum temperature for the growth of the bacteria, this was not true and resistant forms may have arisen.

As the bacteria proved more sensitive to temperature changes than the phage, it became necessary to resort to thermal inactivation tests to find any measurable effect of temperature on the phage. The inactivation of phage by heat has not been constant in tests on phages of plant pathogens. The temperature of inactivation varied from 60° to 85° C. for 10-minute exposures (26, 39, 44). The *Pseudomonas tumefaciens* phage was reported as being inactivated at 70° C. and at 85° C. in 10 minutes (30, 44).

In determining the thermal inactivation of the phage, a 48-hour phage culture was filtered, 5 ml. of the filtrate was placed in each of a number of small, sterile, thin-wall test tubes, and the tubes placed in constantly stirred water baths held at 11 temperatures from 50° to 98½° C. Two tubes were subjected to the heat of the water bath at each temperature, while a thermometer placed in a similar test tube containing an equal amount of distilled water, served to test the temperature of the phage filtrate. The filtrate was held in the water bath for 10 minutes after reaching the desired temperature, removed and quickly cooled, and the filtrate used as inoculum for the usual series of tests for presence of the phage. From the results (Table 2), recording 4 of the 6 tests conducted, it was evident that the phage

TABLE 2.—*The thermal inactivation of Pseudomonas tumefaciens phage*

Test No.	Inoculum	50° C.	55° C.	60° C.	65° C.	70° C.	75° C.	80° C.	85° C.	90° C.	95° C.	98½° C.
1	Phage .....	-	-	-	-	-	+	+	+	+	+++	+++
	Phage check..	-	-	-	-	-	-	-	-	-	-	-
	Bacteria check	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
2	Phage .....	.....	.....	-	-	±	±	±	++	+	++++	
	Phage check..	.....	.....	-	-	-	-	-	-	-	-	-
	Bacteria check	.....	.....	++++	++++	++++	++++	++++	++++	++++	++++	
3	Phage .....	.....	.....	-	-	-	+	±	+	±	++++	++++
	Phage check..	.....	.....	-	-	-	-	-	-	-	-	-
	Bacteria check	.....	.....	++++	++++	++++	++++	++++	++++	++++	++++	++++
4	Phage + .....	.....	-	±	++	++	-	+	±	+	++	++
	Bacteria .....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
	Bacteria check	.....	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++

- = Culture tubes clear.

± = Clarity of culture tubes doubtful.

+ to +++ = Range of turbidity from 0.05 to 0.20 ml. of barium sulphate in McFarland nephelometer.

resisted a temperature of 90° C. for a 10-minute period, but was destroyed at a temperature of 95° C. for a like period. The same resistance to heat was manifested in the presence of the associated organism in tests run in a similar manner, except that an unfiltered phage culture was used.

It may be noted in table 2 that the phage action above 70° C. was much weaker than below this temperature. This peculiarity was further studied by the determination of the titre of the phage after exposure at each temperature, using 10° intervals. The results of 1 of the 2 tests are recorded in table 3.

The definite weakening noted above 70° C. was apparently the same at all temperatures from 70° to 95° C., where the final inactivation occurred. The phage, supposedly of colloidal nature, had apparently undergone 2 changes at different temperatures, each slightly reducing the action of the phage. If the analogies held to exist between the phage and antibodies

TABLE 3.—*The titration of Pseudomonas tumefaciens phage after exposure at intervals from 50° to 95° C.*

Temperature ° C.	10-1	10-2	10-3	10-4	10-5	10-6	10-7
50 .....	—	—	—	—	—	—	—
60 .....	—	—	—	—	++	++	++
70 .....	—	—	++	+	++	++	++
80 .....	+	±	++	+++	+++	++++	+++
90 .....	++	+	+	—	+++	+++	+++
95 .....	+++	+++	+++	+++	+++	+++	+++

— = Culture tubes clear.

± = Clarity of culture tubes doubtful.

+ to +++ = Range of turbidity from 0.5 to 0.20 ml. of barium sulphate in McFarland nephelometer.

were applicable, the action may have been a double dehydration, such as that reported in many antibodies and proteinaceous materials similar to antibodies (40). Whether the lytic principle did undergo a double dehydration, or the phage used was actually composed of 2 portions, having different heat-inactivation points, requires further study. The solution is dependent on the determination of the exact nature of the particles as of phage, or phage absorbed, on a protein carrier. The apparent double nature is considered an important point in considering the nature and use of the phage.

Attempts to separate the phage into strains on the basis of the difference in plaque size do not support the idea of the phage being of 2 parts.

*Titre.* Having shown a reduction in the strength of the phage by heating above 70° C., the normal titre of the phage was investigated. This determination of the potency of the phage was necessary in order to make sure that the dilutions required to eradicate toxic effects of the test material did not at the same time inactivate the phage. The filtrate of a 48-hour phage culture was submitted to the usual bacteriological tenfold-dilution procedure. The tests were run in duplicate, series 1 and 3 being run for each tube to determine the lytic action at each dilution. The data of 5 tests of this nature are recorded in table 4.

The dilution tests all gave the highest titre of the phage at  $10^{-8}$  to  $10^{-11}$ . In the first test reported, the phage action was found in only 4 of the 8 tubes inoculated at the  $10^{-8}$  dilution, while in later tests the lytic action was found at a dilution of  $1 \times 10^{-11}$ .

In dilution tests run by the plaque method, the highest dilution at which plaques were found was  $1 \times 10^{-8}$  and in this case only 1 plaque was found in 1 of the 8 plates.

The results recorded in table 5 show the titre of the phage at various periods from the time of inoculation of the phage. All readings were made at 72 hours. A test was made at 0 hours as a control, since the inoculum

TABLE 4.—*The extent to which the filtrate containing the phage of Pseudomonas tumefaciens may be diluted before losing its lytic power*

No. of test	Age in hours	10-1	10-2	10-3	10-4	10-5	10-6	10-7	10-8	10-9	10-10	10-11	Ck.
1	24	—	—	±	+	+	+	+	+	+	+	+	+
	48	—	—	—	—	—	—	—	—	++	++	++	++
	72	—	—	—	—	—	—	—	—	+++	+++	+++	+++
	96	—	—	—	—	—	+	+	—	+++	++++	++++	++++
2	72	—	—	—	—	—	—	—	—	—	±	+++	+++
3	72	—	±	±	—	—	—	—	—	—	—	+	+++
4	72	—	±	—	—	—	—	—	—	—	+	+++	+++
5	72	±	—	—	—	—	—	—	—	++++	++++	++++	++++

— = Culture tubes clear.

± = Clarity of culture tubes doubtful.

+ to +++ = Range of turbidity from 0.05 to 0.20 ml. of barium sulphate in McFarland nephelometer.

was diluted to 1/100,000 for the original inoculation. From the test it appears that the phage apparently had not increased at 12 hours, and did not reach its full strength until 48 to 72 hours. The conception of the phagic action, a preliminary decrease in titre, followed by a sudden increase when supposedly the bacterium was lysed and the phage particles were released, may be an explanation. The titre was as great at 120 hours as at any other time.

The results indicate that the titre of the phage used varied, depending upon conditions not yet completely elucidated, from  $10^{-8}$  to  $10^{-10}$ , which agreed with other results (18, 30), although far below Muncie and Patel's report (44). The retention of maximum titre up to 120 hours indicated that the phage might not require frequent filtering to keep its potency.

*Longevity.* The retention of lytic capabilities by the phage when stored apart from the associated bacteria is a further indication of its stability, and its resistance to slow oxidation on exposure to the air. If the phage may be successfully stored apart from the bacteria, then the decrease or disappearance of phage in cultures with bacteria must be because of some reaction between the two.

The retention of the lytic power by a 48-hour filtrate of a cleared culture was tested by placing the filtrate in sterile test tubes and making phage tests at intervals. The results recorded in table 6, for the 2 tests conducted by making tests at weekly intervals, showed that the phage was still very active after 63 days. Further tests, 91 days after filtration, were made when the phage solution had dried in most of the tubes. Peculiarly, the tube that was dry showed a very weak lytic action, while that containing a very small amount of liquid showed no such action.

As further evidence of this keeping quality a test was run on a culture that had been placed unfiltered at 5° C. and kept there for 304 days. In this

TABLE 5.—*The titration of the Pseudomonas tumefaciens phage at 12-hour intervals in its development*

Age of phage in hours	10-3	10-4	10-5	10-6	10-7	10-8	10-9	10-10	Check
0 .....	±	++	+	+	+	±	+	+	++
12 .....	++	++	++	++	++	++	++	++	++
24 .....	±	-	-	±	±	±	+	++	++
36 .....	-	-	-	-	-	+	±	++	++
48 .....	++++	±	±	±	-	-	-	+++	+++
60 .....	-	-	++	+	-	±	+	++	++
72 .....	-	-	-	-	-	-	+++	+++	+++
84 .....	+	-	-	-	-	-	-	-	++
96 .....	-	-	±	-	±	-	±	±	++
108 .....	+	+	+++	+	+	+	+	++	++
120 .....	-	-	-	-	-	±	-	-	++

- = Culture tubes clear.

± = Clarity of culture tubes doubtful.

+ to +++ = Range of turbidity from 0.05 to 0.20 ml. of barium sulphate in McFarland nephelometer.

test the phage remained active. It seems that the phage will not deteriorate in less than 300 days when kept in liquid solution in a cold room. Other tests showed the phage still active in a culture kept at 5° C. for over 25 months. In the last test, however, the phage was not the same strain as that used above.

These tests were all conducted with filtrates stored in test tubes plugged with cotton. Drying, except in the cold room, took place rather rapidly. Since the phage seemed to remain active as long as the solution did not dry too much, it seemed that sealed tubes might well keep the phage in an active condition indefinitely.

Further attempts to preserve the phage were carried out by drying under various conditions. The dried phage was taken up in either distilled water or Chester's bouillon by soaking for 6 hours and this solution was sterilized and used as the phage inoculum. In the first test, table 7, the

TABLE 6.—*The phage activity of bacteria-free filtrates tested at various ages after storage in test tubes on the laboratory desk at 23-26° C.*

	Age of filtrate in days										
	0	7	14	21	28	35	42	49	56	63	91 wet 91 dry
Phage	-	-	+	-	±	+	-	+	+	-	+++
Bacteria	++++	++++	++++	++++	++++	++++	+++	+++	++++	+	+++
Phage	-	-	-	±	+	-	-	±	±	±	.....
Bacteria	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	.....

- = Culture tubes clear.

± = Clarity of culture tubes doubtful.

+ to +++ = Range of turbidity from 0.05 to 0.20 ml. of barium sulphate in McFarland nephelometer.

phage filtrate was dried in a vacuum oven at 30 in. of mercury and during the drying the temperature of the oven reached 73° C. The test was negative. Since the thermal inactivation tests indicated a marked drop in phage activity when heated above 70° C., it was thought that the temperature might have been responsible for the failure of the test.

Four tests were run in which the phage was dried in the oven at 30 in. of mercury and a temperature of 50° to 60° C. The results of 3 of these tests are recorded in table 7, and they show that under the conditions at which the temperature did not injure the phage it retained its activity on drying. Tests in which the bacteria were run in control tubes and dried with the phage tubes showed no evidence of phage action, as seen in the table. How long the phage may be kept in this condition is not yet known (24). As mentioned above, however, it appeared to lose its activity when the sterile filtrate was allowed to dry slowly at room temperature, a reaction not yet explained.

TABLE 7.—*The activity of phage filtrates after drying in a vacuum oven at 30 in. mercury at temperatures below 75° C.*

Test No.	Temperature	Phage	Phage control	Bacteria control
1 .....	73° C.	+++	—	+++
2 .....	60° C.	+	—	+++
3 .....	55° C.	—	—	+++
Dried phage .....	58° C.	—	—	+++
Dried bacteria.....	58° C.	+++	—	+++

— = Culture tubes clear.

± = Clarity of culture tubes doubtful.

+ to +++ = Range of turbidity from 0.05 to 0.20 ml. of barium sulphate in McFarland nephelometer.

*Purification.* These tests were all conducted on the *Pseudomonas tumefaciens* phage in bouillon, and it has been observed that the component of liquid media influence the reaction of the phages to some extent (4, 7, 8). The exact nature and properties of the phage will be proved only when it is possible to test these relations on a pure solution of the phage. Two possible methods for separating the phage from extraneous material have been employed, i.e., differential solubility and precipitation.

The solubility of the phage in ether was tested by covering a number of tubes of phage filtrate with 5 ml. of ether, placing in an incubator, and testing the ether extract at intervals by evaporating it over sterile bouillon used as inoculum for determination of the titre of the extracted phage. The residue was similarly tested by dilution tests. The results of typical tests of this type are recorded in table 8. There seemed to be no regularity in the tests unless the temperature of extraction was considered. As may be noted

TABLE 8.—*The titre of ether extract and residue of Pseudomonas tumefaciens phage after extraction with ether for varying periods*

No. of test	Inoculum	Temperature of extraction in ° C.	Number of days extraction					
			0	2	4	6	8	10
1	Residue .....	25	10 <sup>-9</sup>	10 <sup>-9</sup>	10 <sup>-9</sup>	10 <sup>-7</sup>	10 <sup>-7</sup>	10 <sup>-9</sup>
1	Extract .....	25	.....	10 <sup>-6</sup>	10 <sup>-7</sup>	10 <sup>-7</sup>	10 <sup>-7</sup>	10 <sup>-9</sup>
2	Residue .....	5	.....	10 <sup>-8</sup>	10 <sup>-7</sup>	10 <sup>-9</sup>	+	.....
2	Extract .....	5	.....	0	10 <sup>-3</sup>	10 <sup>-1</sup>	±*	.....
3	Residue .....	5	10 <sup>-9</sup>	10 <sup>-9</sup>	.....	10 <sup>-9</sup>	.....	10 <sup>-9</sup>
3	Extract .....	5	.....	0	.....	10 <sup>-8</sup>	.....	0
4	Residue .....	25	10 <sup>-9</sup>	10 <sup>-9</sup>	10 <sup>-4</sup>	.....	10 <sup>-9</sup>	10 <sup>-9</sup>
4	Extract .....	25	.....	10 <sup>-2</sup>	10 <sup>-5</sup>	.....	10 <sup>-5</sup>	10 <sup>-5</sup>

\* = No dilution test made on this date.

+ = Strong phage activity.

± = Phage activity doubtful.

from the table, the phage was apparently soluble in ether at 25° C. but not at 5° C. Other tests reporting ether extraction of phage (35) have been carried out only at the higher temperatures. No explanation can be offered for this apparent decrease in solubility.

Extractions also were conducted with phage dried in the vacuum oven at 30 in. of mercury and at 60° C., by covering the dried material with 10 ml. of the solvent for 24 to 48 hours. The solutions were filtered and dried and the dried extract and residue were taken up in 10 ml. of bouillon. This bouillon was then sterilized by filtration and used as inoculum in tests to determine the location of the phage. A bouillon solution of an untreated dried phage served as a control of the phage action. The tests were entirely confirmative throughout and none of the solvents—acetone, butyl alcohol, chloroform, and ether—showed any extraction of the phage as recorded in table 9. In fact, the results would indicate that acetone, ether, and, to a less extent, butyl alcohol destroyed a large portion of the phage, as shown

TABLE 9.—*The lytic action of extracts and residues of Pseudomonas tumefaciens phage after extraction of the dried phage filtrate by various solvents*

Time of reading in hours	Ether ext.	Ether res.	Ace-tone ext.	Ace-tone res.	CHCl <sub>3</sub> ext.	CHCl <sub>3</sub> res.	Butyl ext.	Butyl res.	Phage control	Bact. control
24 .....	++	-	+	++	-	+	+	+	-	+
48 .....	++	-	++	++	++	-	++	-	-	++
72 .....	+++	+	+++	+++	+++	-	+++	++	-	+++
96 .....	++++	++	+++	+++	+++	-	+++	++	-	+++
72 .....	+++	+++	+++	+++	+++	±	+++	+	+	+++

- = Culture tubes clear.

± = Clarity of culture tubes doubtful.

+ to +++ = Range of turbidity from 0.05 to 0.20 ml. of barium sulphate in McFarland nephelometer.



by the rather slight phage action of these residues. This inactivity agreed with the reported findings on the staphylococcal phages (6, 11, 12) etc. Chloroform did not inactivate the *Pseudomonas tumefaciens* phage.

Since extraction of the phage from dried conditions seemed impossible, purification was attempted by precipitating the lytic principle. Precipitation of the phage was attempted by saturation with ammonium sulphate and with neutral lead acetate, following the method of Vinson and Petre (56). No precipitation was obtained with ammonium sulphate. The lead-acetate method consisted of precipitation of the extraneous materials in 500 ml. of solution with 15 ml. of basic lead acetate, centrifuging and precipitating the active materials in the supernatant fluid with 35 ml. of neutral lead acetate. This precipitate, collected by centrifuging, was washed in 500 ml. of distilled water, twice with 800 ml. of primary potassium orthophosphate, and then twice with 500 ml. of a mixture consisting of equal parts of primary and secondary potassium orthophosphate mixed with 4 times its volume of water. The precipitate was shaken at intervals for 2 to 4 hours and then frozen overnight. The frozen mixture was melted, centrifuged and the supernatant liquid should have contained any active materials that had been precipitated by the neutral lead acetate.

The first test conducted by this complete method was positive, the second negative. In the third test half quantities of reagents were used on 150 ml. of nonfiltered phage solution, separated into 4 portions so that  $\frac{1}{2}$  quantities were used in each of 4 tests. The final solution was adjusted to the same volume as that of the original phage solution. The frozen material was melted quickly by placing in warm water and, since the solution was fairly clear, was filtered through an L3 filter and used as an inoculum. A separate test was made for each of the 4 portions; the results in table 10

TABLE 10.—The lytic action of precipitates of *Pseudomonas tumefaciens*-phage filtrates obtained by neutral lead-acetate precipitation according to the method of Vinson and Petre

Inoculum	A	B	C			
			1	2	3	4
Phage .....	-	+++	+++	-	+	±
Phage control .....	-	-	-	-	+	±
Bacteria control .....	+++	+++	+++	+++	+++	+++

- = Culture tubes clear.

± = Clarity of culture tubes doubtful.

+ to +++ = Range of turbidity from 0.05 to 0.20 ml. of barium sulphate in McFarland nephelometer.

indicate a great variation in the 4 tests, although all showed some phage in the final solution.

The dilution, considering that the precipitates were not dried, of any liquid phage remaining adsorbed on the precipitate at each treatment would be somewhat greater than that at which the phage lost its activity. The tests might, therefore, be deemed inconclusive. Considering the strength of the lytic action obtained in some cases, it was considered that the precipitation of the phage by the methods used in plant-virus research was possible.

The evidence as to solubility of the phage in organic solvents agreed with the general work on phages, although the precipitation tests indicated that this phage was much more resistant to precipitation than usual. This may have been because of its much lower adsorption on colloidal or other adsorptive material.

#### SOME CHEMICAL CHARACTERISTICS OF THE PHAGE

The phages probably belong to the ultramicroscopic group of agents that include the viruses of plants and animals. In the designation of the characters of these principles, their resistance to treatment by certain chemicals has been tested. It was considered that the resistance of the phages to similar treatments should, therefore, prove advantageous in the characterization of these lytic principles.

*Alcohol.* Alcohol has been reported as having different effects on phages, varying from a solvent action (35), to precipitation and inactivation (4, 8). The precipitation of the phage of *Pseudomonas tumefaciens* by alcohol was tested first. The strength of the alcohol in 40 ml. of phage filtrate was increased from 10 per cent to 90 per cent without obtaining any evidence of precipitation in 48 hours, in 3 tests.

The phage filtrate then was incubated with varying concentration of alcohol for 1 to 6 hours and the phage identification tests performed. One ml. of the filtrate was added to 9 ml. of alcohol so diluted with bouillon that the final 10 ml. of solution possessed the required dilution of alcohol. After the proper incubation the solution was diluted 1 to 1000 and used for the inoculum in the tests.

When left in contact with the alcohol for one hour, the phage was still active in the solution containing 95 per cent alcohol. After 6 hours' exposure to the alcohol, the *Pseudomonas tumefaciens* phage retained a slight activity in the 70 per cent alcohol exposure, but was destroyed by the higher concentrations. The test was repeated 5 times in liquid culture and 3 times by the plaque method. The results were the same in all but one of the plaque tests, where no activity was obtained after exposure to 70 per cent alcohol for 6 hours.

The resistance of the phage to alcohol was comparable to that shown by most viruses, and exceeded that of the associated bacteria, which were inhibited by exposure to 60 per cent alcohol for 1 hour.

*Hydrogen peroxide.* The loss of action of phages on drying has been considered to be due to their slow oxidation. Lominski (36), using this as a starting point, found that the phage associated with staphylococci could be destroyed by oxidation without destruction of the bacteria. On the other hand, LeMar and Myers (34, 35) oxidized autolysed bacteria in order to produce an artificial lytic principle. It seemed advisable, therefore, to test the action of hydrogen peroxide on the phage of *Pseudomonas tumefaciens*.

Using the methods described in the alcohol tests, the phage was exposed to 1 per cent hydrogen peroxide for 72 hours. This solution was diluted 1 to 1000 and used as the inoculum in identification tests. The results reported in table 11 summarize the 3 trials made, and indicate that, although

TABLE 11.—*The lytic power of the phage after 72 hours' exposure to one per cent hydrogen peroxide and one hour exposure to dilutions of phenol*

Treatment of phage used for inoculation	Hours from inoculation				Hours exposure
	24	48	72	96	
Hydrogen peroxide .....	+	++	—	++	72
Untreated .....	+	—	—	+	72
Bacterial control .....	++	++	...	+++	—
1/10 phenol .....	+	++	+++	+++	1
1/20 " .....	+	++	+++	+++	1
1/30 " .....	+	++	+++	+++	1
1/40 " .....	+	—	±	—	1
1/50 " .....	—	—	—	—	1
Bacterial control .....	+	++	+++	+++	—

— = Culture tubes clear.

± = Clarity of culture tubes doubtful.

+ to +++ = Range of turbidity from 0.05 to 0.20 ml. of barium sulphate in McFarland nephelometer.

the phage was able to resist this exposure to the oxidizing action of hydrogen peroxide, it apparently was slightly reduced in activity, as indicated by the delay in clearing.

*Phenol.* Another reagent commonly employed in determining the properties of most phages of animal pathogens is phenol. The retention of activity of the phage after exposure to varying concentrations of this reagent has been tested since it has been used as the standard in work on bactericides.

The action of phenol on the phage of *Pseudomonas tumefaciens* was studied as described for the alcohol tests, except that distilled water was used as the diluent, because phenol precipitated the bouillon. The exposures were for 1 hour, and tests were performed as in the alcohol tests. The results in table 11 are summarized from 3 such tests and include only the stronger solutions. All the weaker solutions disclosed a marked resis-

tance on the part of the phage. These results show that the phage was incapable of withstanding phenol at dilutions of less than 1/40. This agrees with the work on animal-pathogen phages, where the concentrations resisted varied from 1/100 to 1/40. This strength of phenol apparently lowers somewhat the activity of the phage as noted by the delay in the development of the lysis. In the check tests, employing the susceptible bacteria in the absence of the phage, a slight growth occurred after exposure to 1/160 phenol, but none after exposure to stronger solutions. Thus again the phage resisted the action of much stronger solutions than the associated bacteria.

*Hydrogen-ion.* Although phenol is mildly acidic, it is not used in determining the resistance to hydrogen-ion activity, because it is toxic. The effect of hydrogen-ion activity on the lysis of the phage is concerned in the reaction of the medium in which the phage tests are being performed, in storage relations of the phage, as well as in the concentrations of acid and alkali that may be withstood by the lytic principle.

Two preliminary tests on the effect of hydrogen-ion activity on the phage were conducted on media adjusted to unit pH values from pH 3 to pH 9. The phage activity, bacterial growth, and pH of the media after sterilization are recorded for one of these tests in table 12, using the 48-hour readings.

TABLE 12.—*The influence of hydrogen-ion activity on the production of lysis of Pseudomonas tumefaciens by its phage*

	Hydrogen-ion activity (pH)						
	3	4	5	6	7	8	9
Phage .....	—	—	—	—	—	—	—
Bacteria control .....	—	—	++	++	+++	++	+
pH test .....	3.0	4.1	5.1	6.2	6.8	7.6	8.0

— = Culture tubes clear.

+ to +++ = Range of turbidity from 0.05 to 0.20 ml. of barium sulphate in McFarland nephelometer.

It was evident that the phage was active at all hydrogen-ion activity values that allowed the growth of the associated organism.

As this method was not sufficiently extensive to show the full hydrogen-ion relations, further tests were made by the same methods as those used in the chemical tests. The values were determined as dilutions, as employed in plant-virus research (32), rather than pH determinations. The tests were made with nitric acid and sodium hydroxide diluted with Chester's bouillon. The results recorded in table 13 are only those obtained in the 3 tests at the critical dilutions. The other tests were negative or positive depending on whether they were lower or higher dilutions.

TABLE 13.—*The lytic power of the Pseudomonas tumefaciens phage after one hour's exposure to various concentrations of acid and alkali*

Dilution of nitric acid	Phage	Phage control	Bact. control	No. of tests	Dilution of sodium hydroxide	Phage	Phage control	Bact. control	No. of tests
1/100	++	—	++	2	N/8	+++	—	+++	3
1/200	++	—	++	2	N/16	+++	—	+++	3
1/2000	+++	—	+++	3	N/32	++	—	++	3
1/3000	+	—	+++	3	N/64	—	—	++	3
1/4000	+	—	+++	3	N/128	—	—	++	3
1/5000	—	—	+++	3					

— = Culture tubes clear.

+ to +++ = Range of turbidity from 0.01 to 0.20 ml. of barium sulphate in McFarland nephelometer.

The results in table 13 indicate that the phage did not resist the action of nitric acid (sp. g. 1.502) stronger than the 1/3000 dilution. The tests were run by the liquid and plaque methods and as the two methods agreed only the former was reported. The phage was thus much less resistant to acids than many viruses, some of which have been found to withstand nitric acid at strengths of 1/200 (32).

The resistance of the phage to alkali, sodium hydroxide, is reported in table 13, also. From this it may be seen that the phage was destroyed by N/32 sodium hydroxide when exposed to its action for 1 hour. As in the case of the acid action, tests by the liquid and plaque methods agreed and only the former were reported. The phage exhibited a lower resistance to alkali, also, than that shown by many viruses.

It appears that the phage was more easily destroyed by solutions either strongly acid or alkaline than many plant viruses, although it resisted stronger solutions than the associated bacteria.

#### SOME BIOLOGICAL PROPERTIES OF THE PHAGE

The ultimate aim of investigations on the nature of the interactions of 2 factors is to explain the nature of this action on the basis of a known physical or chemical phenomenon. However, since the actual constitution of many of the reactions of phages with their associated bacteria will remain unknown for some time, these properties must be catalogued as of a biological nature. The location of the phage in the plant, the specificity of the phage actions, etc., undoubtedly have a physical or chemical explanation, at present unknown; but these properties can, nevertheless, be more thoroughly understood only when sufficiently investigated from a biological standpoint.

*Location of the phage in plants.* Although a systematic search for the phage has not been made, the 41 isolations made to date do indicate where

the phage may be found in some of the hosts of *Pseudomonas tumefaciens*. In 21 isolations from galled tomatoes, 14 were positive; 2 were positive in 3 tests from sugar beets; 2 positive isolations were made from Marguerites; while 2 castor-bean galls, 2 one-year-old Peach galls, and 1 Bryophyllum gall gave negative results. The remaining 10 isolations, from healthy plants, were all negative.

In these isolations, tomato galls have been used at ages varying from 20 days to 4 months from the date of inoculation, and all attempts to trace the development of the phage in the plant indicated that it was present as soon as gall formation started. There was no indication of the appearance of the phage being associated with the breakdown of the host cells. Isolations were always made from live tissues.

In the sugar beet, Marguerite, and all plants showing negative results, the isolations were made from the galls themselves. However, in the tomatoes, where the bacteria may be found in the xylem (46, 48, 50), in part of the plants which show no galls, isolations were tried from internodes above the galls. In the 4 cases where internodal isolations were tried above galls which yielded a phage, the phage always was found in the stem at 3 and 6 in. above the gall. In the 3 cases where the galls gave negative results, the internodal isolations also gave negative results.

The failure to obtain the phage in certain galls was attributed to temperature action in view of Riker's observation (47) that growth of galled tissues did not occur above 30° C., since it was considered that the phage originated within the living tissues of the galls.

It was evident that the phage, when present in galls on tomato plants, was present also in the healthy portions of the stems, as far as 6 inches away from the gall. The isolation of the phage from the healthy portion of galled plants agrees with the other work reported on crown-gall as a source of phage (10, 16, 18, 30, 44).

*Specificity of the lytic action.* The isolation of a phage from a given plant will depend primarily on culturing it on a strain susceptible to its action. This action of the phage on a number of strains and organisms should be known, also, to enable correct designation of the phage, to determine any peculiarities of action on different organisms, to enable more exact comparisons with other phages, and to widen the field for examination of its practical application.

The phage under consideration was tested on *Pseudomonas tabacum* (two strains, 257 and 258 in table 14), *Ps. campestris* (B2802), and *Bacterium stewartii* (S15) and found to be without action.

As preliminary tests had indicated the phage might be active only on pathogenic isolates from galls, it was tested on a number of isolates of *Pseudomonas tumefaciens*, obtained from Dr. G. L. McNew, to whom my

sincerest thanks are due. The majority of these isolates were made from the susceptible culture originally used with the phage (B902) after purification by a series of 5 single-colony isolations. The isolates were selected for their wide range in pathogenicity and included a rough highly pathogenic form. The collection also included a strain originally obtained from K. D. Butler and isolated from cottonwood at Tucson, Arizona, a strain from G. Harrar, which was isolated from apple at Ames, Iowa, and a strain originally isolated by C. W. Hungerford.

The specificity tests were run as a normal phage test by employing the test bacteria as the bacterial seedlings used for the culture of the phage. These tests were repeated at least twice. As recorded in table 14 they indi-

TABLE 14.—*The reaction of 15 isolates of Pseudomonas tumefaciens and of other organisms to tests of pathogenicity on tomato, relative growth rate, and susceptibility to and adsorption of Pseudomonas tumefaciens phage*

Culture	Susceptibility to phage	Adsorption of phage	Pathogenicity of tomato	Relative rate of growth
B91 .....	++++	.....	++++	++++
B902 .....	++++	++++	++++	++++
B912 .....	++++	+++	.....	+++
B44 .....	+	±	++	++++
B46 .....	+++	±	++	++++
B47 .....	-	-	++	++++
B48 .....	++++	-	++++	++++
B49 .....	++++	-	+	++++
B50 .....	-	-	-	+
B51 .....	-	-	++	+
B52 .....	-	-	++	+
B53 .....	+	-	++	+
B54 .....	-	±	-	+
B55 .....	-	±	-	+
B56 .....	++++	++++	++++	++++
B57 .....	-	.....	-	+++
B58 .....	-	.....	-	+++
B2802 .....	-	.....	-	++++
S15 .....	-	.....	-	++++

+++ Indicates highest degree, and - lowest degree of reaction to test considered.

cate a variable result in the susceptibility of the different strains. There seemed to be little correlation between the other tests conducted on these strains and their susceptibility to lysis. However, all the strains that were highly pathogenic and were isolated from tomato showed susceptibility, and none of the strains showing a lack of virulence on the tomato were susceptible. The strains showing intermediate pathogenicity on tomato varied in their relations, although none showed a great deal of susceptibility. It is interesting to note that culture B46, isolated by Harrar from apple at Ames, Iowa, was susceptible, while the culture B44 from Hungerford was only slightly so. The culture isolated from cottonwood in Tucson, Arizona, was

not susceptible to the phage action. The rough strain, isolated from the original susceptible strain and found to be severely pathogenic, was also very susceptible to the action of the phage.

*Adsorption of the phage by bacteria.* Adsorption of the phage by the associated bacteria is said to be the first step in the lytic process, being a character in some cases of dead bacteria that are otherwise resistant to the action of the phage (13, 15, 20, 22). Tests were conducted, therefore, to see if the differences in the adsorption of the phage might explain its specificity.

The first investigations were carried out on dead bacteria of the standard strain B902 to determine if they adsorbed the phage and rendered it inactive. Two trials, run by the plaque method, under the assumption that each particle of phage produces a plaque, indicated a slight adsorption. The difference, however, was too small to be conclusive.

This adsorption test was repeated by inoculation of a suspension containing 250,000,000 live bacteria per ml. of strain B902 with phage such that its strength was 1/2,000,000 of normal during the exposure. Transfers were made from the cultures at hourly intervals from 5 to 9 hours after inoculation, as this period had been shown to overlap the beginning of the logarithmic phase of phage growth, and, if adsorption occurred, it should have been evident by that time. In order to test whether the phage was actually adsorbed on the bacteria, transfers were made from cultures filtered through sterile L3 filters and from nonfiltered culture tubes. Tests also were made of the phage to see if numerous resistant organisms were present.

The results recorded in table 15 indicated that there was a slight difference in the number of phage particles between the filtered and nonfiltered inocula. The increase appeared to have taken place at different times in the 2 test types and over a 2-hour period. Since the nonfiltered material plates were completely lysed after 6 hours, no quantitative interpretation was possible. It appeared that filtering off the bacteria reduced the titre of the phage.

Since the plaque method was found to be so unreliable, a method was adopted using the bouillon tubes as tests of phage activity. A suspension of bacteria made from a 48-hour agar culture was heated for 1 hour at 70° C., and after adding 1 ml. of suspension to each of a series of tubes containing 9 ml. of Chester's bouillon, the suspensions were again heated for one hour at 70° C. To this series of suspensions of dead bacteria the phage was added in a series of dilutions ranging from  $10^{-2}$  to  $10^{-9}$ . The tubes were incubated for 16 hours and tested by the regular phage identification series. A control was run by incubating the same dilutions of phage in sterile Chester's bouillon that had been heated with the bacterial suspensions. Adsorption would be indicated by a drop in titre of the phage in



the tubes containing the bacterial suspensions. As adsorption was found in the tests of B902, the tests were conducted with the 14 other strains of *Pseudomonas tumefaciens* showing ranges of pathogenicity from nonvirulent (B50, B54, B55) to an extremely pathogenic strain, B48, capable of producing very large galls in 6 weeks. All tests were repeated at least twice. The results, (Table 14) indicated that few if any of the bacterial

TABLE 15.—*The adsorption of phage by living bacteria as shown by filtered and nonfiltered inocula (plaque counts)*

Age, in hours	Phage	Filtered	Nonfiltered
5 .....	+	11	33
6 .....	++	34	∞
7 .....	∞	24	∞
8 .....	∞	108	∞
9 .....	∞	∞	∞

+ = A few bacterial colonies.

++ = A number (200) well-scattered bacterial colonies.

∞ = The plate was cleared by the phage.

Numerals are the number of plaques per plate.

strains so adsorbed the phage as to inactivate it.

As it was noticed in these tests that B902, when run as a control for adsorption tests, occasionally did not react positively, it was thought that the adsorption did not inactivate the phage. An attempt, however, was made to determine whether adsorption did inactivate the phage. The methods were the same as those used before, but tests were made before and after filtering the tubes containing the lower dilutions of the phage. These results (Table 16) indicate that, since the filtered material rather con-

TABLE 16.—*The adsorption of phage by dead bacteria as shown by filtered and nonfiltered inocula (liquid tests)*

Age, in hours	Nonfiltered	Filtered
0 .....	+	+++
1 .....	+	—
2 .....	+	++
3 .....	—	++
4 .....	—	++
5 .....	—	+
6 .....	—	—
7 .....	+	+++
8 .....	—	—
9 .....	—	++
10 .....	—	No test

+ = The titre is  $10^{-1}$  less than control.

++ = The titre is  $10^{-2}$  less than control.

+++ = The titre is  $10^{-3}$  less than control.

— = Titre equal to or greater than control.

sistently gave a lower titre than the nonfiltered and control materials, which agreed rather closely, the phage was absorbed on dead bacteria, that this adsorption was not, apparently, very definite nor lasting, and that it inactivated the phage only when the phage-bacteria complex was removed from the inoculum.

Adsorption of the phage then occurs to a slight extent on both living and dead bacteria, but not to the degree of making the phage incapable of producing lysis of other organisms left in the same solution. The tests on the adsorption of the phage are, therefore, of doubtful value and do not account for the specificity of the phage. The specificity observed is similar to that of Izrail'skii (30), being broader than that found by Muncie and Patel (44), although not of a polyvalent nature, as was that investigated by Coons and Kotila (18) and Brown and Quirk (10).

*The protective value of the phage in plants.* The phage, although active only on certain strains of the associated organism, was found to possess characters indicating that it might serve a protective rôle in a manner similar to the destruction of legume bacilli attributed to their phage. The results obtained thus far indicated that the phage, much diluted, was capable of causing dissolution in a rather densely seeded culture of bacteria susceptible to its action and that the phage would resist most conditions under which these bacteria were found. It also had been shown that where it was impossible to isolate a phage from stock cultures it might be obtained by the correct treatment from a gall produced by this culture, or from the healthy portion of the plant bearing this gall. The conclusion must be then that the phage arose in some manner within the plant.

If the phage arises in the plant, is it in some measure responsible for the failure of galls to spread? Can the phage be introduced into the plant and protect it from the action of the bacteria?

The first question cannot now be answered except to indicate that, although contrary to some reports (2, 25, 38, 49, p. 177), the results of numerous attempts to produce galls by inoculation at the edge of older galls have all resulted in the appearance of galls from these secondary inoculations. The formation of galls on a second inoculation has been found by other workers (9, 45, pp. 139-141, 47). Hence, such protection by natural phage was not strong enough to protect from artificial inoculation by hypodermic needles.

The second question has been investigated more fully. In a number of tests phage filtrate was introduced into the plant by hypodermic inoculation and by placing plants, with the roots cut off under water, in phage filtrate and allowing the phage to be drawn up in the vascular system. The plants were inoculated, immediately or after 7 to 14 days, with a pathogenic strain of *Pseudomonas tumefaciens* susceptible to the phage. In no case

was any evidence obtained of protection by the phage introduced into the plant prior to or at the time of inoculation by the usual hypodermic method.

Inoculations were carried out also with the phage in an attempt to confirm the supposition that the lysis of bacterial cultures by the phage would prevent the formation of galls. After a series of preliminary trials, 2 experiments were run testing the phage culture by making inoculation into 3- and 4-week-old tomato plants at 12-hour intervals. In a typical test, reported in table 17, tomatoes were inoculated, 5 plants with the phage and 5

TABLE 17.—*The effect of bacteriophage on the pathogenicity of Pseudomonas tumefaciens, as determined by inoculations of tomatoes with phage cultures made at twelve-hour intervals*

Inoculum	0	12	24	36	48	60	72	84	96	108	120	132	144	156	168
Phage .....	++	+	0	±	±	±	±	±	±	±	+	+	+	+	+
Bacteria .....	+++	++	+++	+++	+++	+++	+++	+++	++	+++	+++	+++	++	+++	+++
Phage filtrate	0														
Phage (plant)		0	0	+	+	0	±	+	++						
Phage (plant)	250	100	1	4	12	2	1	100	100						

0 = No galls formed.

± = Small swellings.

+ to +++ =  $\frac{1}{4}$ " ranges in size of galls at six weeks from inoculation.

with a bacterial culture growing in Chester's bouillon, at each 12-hour interval for 168 hours. The results show that, although the phage at 0 hours produced normal galls, the 12-hour phage culture failed to produce galls. This was true of the inoculations for all periods up to 168 hours, although in the later periods noticeable galls were produced. The cultures were apparently slowly developing the secondary cultures. As these tubes were held on the shelf unshaken, this secondary clouding was slow in developing.

Other cultures, which were shaken at 12-hour intervals, when readings were made, developed turbidity in 84 hours and inoculations made then, or later, resulted in the formation of normal-size galls. Cultures isolated from these clouded phage passage tubes were tested for pathogenicity and in all cases were highly pathogenic.

Since the phage did not prevent gall formation by a culture unless the phage had been in contact with the bacteria for some period, it seemed doubtful whether the phage could be used for protective purposes, as suggested by some authors.

Investigations as to the period of contact necessary to produce lysis have not been carried out directly for the relation to protective action. In general, the active phages appeared to be adsorbed by the susceptible organism in 10 to 15 seconds, in which case the lysis occurred in from 1 to 2 hours (21). Burnet and Lush (14), however, found that about 1 hour was necessary for complete contact of phage with the bacteria in their work on

TABLE 18.—*The properties of the phage of Pseudo*

Investigators	Source of phage	Cultures lysed	Titre	Thermal in-activation	Temperature range (° C.)	Longevity	Hydrogen-ion effect
Coons and Kotila	Rotted carrot, soil, river water	<i>Bacillus carotovorus</i> <i>B. astrosepticus</i> <i>Ps. tumefaciens</i> ( <i>B. amylovorus</i> ) ( <i>B. typhosus</i> )	$1 \times 10^{-8}$		Min. 7.8 Opt. 25.0 Max. 36.1	5½ mo. in sealed flask	
Izrail'skiĭ	Beet gall	3 out of 9 strains of <i>Ps. tumefaciens</i>	$1 \times 10^{-11}$	70° C.			
Brown and Quirk	Rotted carrot, galls, healthy carrot	<i>B. carotovorus</i> <i>Ps. tumefaciens</i>					Plaques limited to pH 5.6-7.1
Muncie and Patel	Soil, culture, sugar beet gall	Parent strains of <i>Ps. tumefaciens</i>	$1 \times 10^{-21}$	85° C. in 10 minutes			
Chester	Pelargonium and beet gall, healthy portion near gall, healthy beet		$1 \times 10^{-8}$				
Kent	Tomato gall	Highly virulent and some mid-virulent strains of <i>Ps. tumefaciens</i>	$1 \times 10^{-11}$	95° C. in 10 minutes	All temperatures allowing bacterial growth	300 days in test tube at 5° C.	All values allowing bacterial growth

*Staphylococci* phages. In the case of the phage for *Pseudomonas tumefaciens*, Muncie and Patel (44) found that by using the tumor-producing ability of the organisms as a test measure, a minimum exposure of 9 hours was necessary for prevention of gall formation.

From the above tests it would appear that, as in the case of lysis, the minimum time necessary for phage-bacterial contact in order to prevent gall formation depended on the ratio of phage to bacteria at inoculation, environmental conditions, etc. Hence, a time limit can hardly be placed on the relationship, since the loss of pathogenicity and lysis seemed to occur simultaneously. It was definite, however, that prophylaxis was possible only if a definite contact with all the bacteria could be made by the phage before their entry into the host, and it seemed that neither artificially introduced, nor natural phage would protect the host from the action of the gall-producing bacteria.

#### DISCUSSION

The properties of a single strain of phage acting on *Pseudomonas tumefaciens* have been fully investigated. This phage was isolated from a tomato gall and was specific for certain strains of *Ps. tumefaciens*. The properties studied were selected from the usual tests conducted on bacteria

*monas tumefaciens* reported by various investigators

Rapid drying	Alcohol resistance	Phenol resistance	Acid resistance	Alkali resistance	Hydrogen peroxide resistance	Extraction	Secondary cultures	Adsorption by bacteria
							Resistant	
							Rough—turned smooth on further exposure to phage	
Phage resistant if temperature below 60° C.	70% for 6 hours	1/40 phenol for one hour	1/3000 nitric acid for one hour	N/64 NaOH for one hour	One % for 72 hours	Resists organic solvents	Susceptible	Slight, and phage not inactivated

and virus principles in plant pathological work as the most suitable for the designation of the phage. In earlier work on such phages there was no indication that a single phage was used in all the studies. It is evident, however, from the variation in specificity shown by these isolates, (Table 18) that there must exist a number of phages for this organism. The source whence these phages were isolated, soil, water, galls, and rotted carrots, would suggest even more strongly that differences existed among the lytic principles attacking *Ps. tumefaciens*. It is even more obvious from table 18 that few of the properties of any of these phages or phage groups have been investigated. The work of the different authors cannot be combined to supply the deficiencies, as there has been no attempt to use standardized procedures.

The lack of integration and standardization of the investigations is evident, also, in the work on phages of other bacterial plant pathogens. Table 19 presents a summary of the studies on the source and properties of the phages of *Rhizobium* species. This group of phages shows even more clearly the indication that numerous phages, active on these organisms, exist and that there has been little study of their properties. There is introduced here the additional factor of attempting to group phages active on different species of bacteria.

TABLE 19.—*The properties of phages of Rhizobium strains as determined by various workers*

Investigator	Source of phage	Cultures lysed	Resistance to drying	Thermal inactivation	Plaque production	Titre	Effect of hydrogen-ion
Gerretsen, Gryns, Sack and Söhngen	Nodules, roots, stems and soils	Homologous bacterial strain	2 months on filter paper	Serradella strain 60° C. for 30 min. Others withstand 65° C. for 15 min.	Negative		
Grijns [Gryns]	Roots and stems				Positive		
Izrail'skii	Nodules	All races of <i>Rhizobium</i>					
Hitchner	Nodules	Homologous bacterial strains			Negative	1 x 10 <sup>-5</sup>	
Laird	Stock cultures, nodules	Variable			Positive	1 x 10 <sup>-7</sup>	Opt. at pH 7.6–8.0
Demolon and Dunez	Nodules, roots, stems and soil	Variable				1 x 10 <sup>-7</sup>	
Vandecasteele and Katznelson	Stock cultures, soils and nodules					1 x 10 <sup>-11</sup>	

It is felt, therefore, that the present studies, which have been conducted on a single phage isolate while lysing a single strain of susceptible bacteria, have so defined this phage as to allow further isolates to be compared with it. Which of these properties may or may not serve for classification purposes will depend on the nature of other phage strains.

The primary stage of the lytic process was not so definite as found in some cases (13, 15, 17). The phage was adsorbed to the bacteria and in some manner stimulated their lysis. It appeared that the phage either might easily be separated from the adsorbed state or was capable of producing lysis of one bacterium while adsorbed to another. The nature of the lysis, agglutination preceding the complete clearing, might explain this peculiar adsorption relation. If the phage, while adsorbed on a dead organism, can cause agglutination, contact would be gained with living organisms and the lytic stimulation produced. From the resistance shown by the phage to the physical and chemical agents, the stimulation of the lytic process would seem to depend on contact, as the assumption that enzymes or other catalytic substances are produced by the phage, which cause the action at a distance, merely complicates an already obscure picture. Hence, it would appear that the indication that the phage was only slightly adsorbed, if at all, and in that way produced its stimulation, allowed a better understanding of the facts of its action as now known and the results obtained above.

While the lysis was slow, the indication was that no increase occurred in the titre of the phage until 6 to 8 hours after isolation, which again pointed to slower development and action on the part of the phage. Whether less activity of a single particle was indicated or a high number of particles was necessary to stimulate a single cell to be lysed has not been answered. However, both the delay in increase of titre and in lysis indicated that the phage would not serve as a protective measure in the plant, as the bacteria would soon escape the sphere of action of the phage.

This work, therefore, points to 2 lines of work that need investigation in order to settle the question of the value of phages in relation to *Pseudomonas tumefaciens*: First, the determination of conditions actually allowing or hindering beneficial therapeutic action; second, the investigation, following further knowledge of phage-bacteria relations within the host, of the possibility of an artificial production, and thereby an explanation, of the origin of the lytic principle.

#### SUMMARY

Bacteriophages producing lysis of *Pseudomonas tumefaciens* have been isolated from crown gall on tomato, sugar beet, and Marguerite, and from healthy portions of galled tomatoes. Phages have not been obtained from healthy plants.

The properties of a single uniform phage isolate have been determined during 25 months of continuous culturing. The phage was characterized by the agglutination of the bacteria accompanying the lytic action.

The phage had a maximum titre of  $10^{-11}$ , was inactivated by a 10-minute exposure at 95° C., preserved its lytic action on rapid drying at 50° to 60° C., and withstood aging *in vitro*, provided drying was prevented.

The phage retained its lytic action on exposure to 70 per cent ethyl alcohol for 6 hours, 1/40 phenol for 1 hour, and 1 per cent hydrogen peroxide for 72 hours. The action of 1/3000 nitric acid and N/64 sodium hydroxide was resisted during 1-hour exposures.

The phage was not extracted by ether, chloroform, acetone, or butyl alcohol. It was not precipitated by ammonium sulphate, although it appeared to be precipitated by neutral lead acetate.

The phage exhibited specificity toward certain strains of *Pseudomonas tumefaciens*, although this character could not be explained by any of the other properties of the phage.

The phage seemed to have little therapeutic value, and the adsorption onto bacteria was slow, incomplete and did not produce inactivation.

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## SEROLOGICAL STUDIES OF PLANT VIRUSES

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The serological approach to the problem of plant-virus classification has proved a fruitful field of study in recent years. A number of the plant viruses may be identified and classed in groups of closely related strains by means of the precipitin test (2, 3). A modified precipitin method has been described recently, whereby it is possible to identify viruses rapidly in the field with very little equipment (7). A considerable number of plant viruses, however, have regularly failed to give precipitin reactions (6). Part I of this paper is designed to report the progress made up to the present in classifying plant viruses by serological means and the present limitations of the method. A second section is devoted to studies on the techniques of plant-virus serology.

### PART I. SEROLOGICAL EVIDENCE IN PLANT-VIRUS CLASSIFICATION AND THE LIMITATIONS OF THE METHOD

The groups of serologically related viruses, as defined in a previous paper (3), have been subjected to further study with the following results.

*Potato Aucuba-mosaic Viruses.* Juices containing the virus of aucuba mosaic of potato (not to be confused with aucuba mosaic of tomato, which is a strain of tobacco mosaic) have been shown to give a specific precipitin test. Until recently, however, no strains of this virus, other than the typical strain from potato, have been encountered. A few months ago, T. P. Dykstra requested a serological determination of the Canada-streak virus of potato. Juices containing this virus reacted with serum prepared against the aucuba-mosaic virus and antiserum for Canada-streak precipitated aucuba-mosaic juices. Infection tests of the 2 viruses on a number of hosts indicated that they were very similar; hence, it is concluded that Canada streak is a strain of potato-aucuba mosaic.

*Etch Viruses.* According to the precipitin test, etch and severe etch are strains of the same virus group. The rare cross reactions with tobacco mosaic, mentioned in an earlier paper (3), have not been obtained in many repetitions of the test, and, hence, were probably due to a contamination of the cross-reacting materials. Blakeslee's Z-mosaic of *Datura* regularly interacts with the etch-virus sera and, accordingly, is looked upon as a strain of the etch group.

*Cucumber-mosaic Viruses.* The close serological relationship between cucumber mosaic and the veinbanding virus of potato (3) has been confirmed in many experiments involving the viruses of these 2 diseases. A number

of the strains of cucumber-mosaic virus, isolated experimentally by Price (13), when tested by the field precipitin technique, proved to be serologically indistinguishable from cucumber-mosaic and veinbanding viruses. On the other hand, juices containing the viruses of celery mosaic and lily mosaic, both considered by Price to be strains of cucumber mosaic (14, 15), each failed to react with sera for any of the cucumber-mosaic group. The reciprocal tests, i.e., preparation of sera for celery mosaic and lily mosaic and testing these sera against their proper viruses and against cucumber-mosaic juices, have not been performed. Doolittle's cucumber mosaic (characterized by more severe distortion on tobacco than Price's cucumber mosaic) also failed to react with sera for Price's cucumber mosaic or veinbanding.

Of 2 Canadian tobacco viruses received in December, 1936, from G. H. Berkeley, precipitin tests showed one to be an exceptionally mild strain of potato-veinbanding virus, the other a mixture of the veinbanding virus and tobacco ring spot. These results were confirmed by Berkeley's infection tests. An authentic sample of the Y-virus of European workers, kindly provided by T. P. Dykstra, proved to be serologically indistinguishable from the other viruses of the cucumber-mosaic group. Stipple streak of potato, when freed from its usual admixture of potato latent mosaic, likewise reacted as one of the veinbanding group. In potatoes in the field, latent mosaic is almost invariably present, and, accordingly, stipple streak may be looked upon as a type of rugose mosaic (veinbanding plus latent mosaic).

*Tobacco-mosaic Viruses.* Up to the present, plant-virus serology has been confined almost exclusively to the testing of juices of systemically infected leaves and succulent stems. It would be very desirable to have available data on the serological behavior of other virus-infected plant tissues, and in connection with the potato viruses and tuber-indexing a knowledge of the serological activity of virus-infected tubers will be necessary. The writer has had occasion to test for tobacco-mosaic virus antigens in (a) roots grown by White's tissue-culture methods (19) and in (b) leaves of plants in which virus is restricted to local lesions. These 2 types of test resulted as follows.

Tobacco-mosaic and tomato-*aucuba*-mosaic viruses were readily detectable by the precipitin test in the juices of macerated root tissues from tissue cultures containing these viruses. A sample of similar roots infected with latent mosaic of potato gave no reaction, but it was later found that in the many transfers undergone by these roots the virus infection had been outgrown and that the roots were no longer infective.

Tobacco-mosaic virus was propagated in *Nicotiana langsdorffi* Weinm., where it caused local necrotic lesions but no systemic infection under the conditions of these experiments. The infected leaves were macerated and the juice expressed and concentrated by precipitation with ammonium

sulfate. The resultant product gave a strong specific precipitin reaction with tobacco-mosaic-immune serum.

These 2 types of experiment indicate that the heavy-weight protein, isolated by Stanley from systemically diseased tobacco and tomato plants, and responsible for the precipitin reaction, also occurs in considerable amount in local-lesion infections of this virus and in chlorophyll-free root-tissue cultures.

*Serological Grouping of Reactive Viruses.* Precipitin tests up to the present, in this laboratory and elsewhere, have provided evidence that the plant viruses that have given group-specific precipitin tests may be classified as follows:

a. *Tobacco-mosaic group:*

Field-type tobacco mosaic (Johnson's tobacco virus 1).  
Holmes' attenuated and masked strains of tobacco mosaic.  
Jensen's yellow and necrotic tobacco-mosaic isolates.  
Aucuba mosaic of tomato.  
Johnson's tobacco virus 6.  
Certain Belgian necrotic diseases of tobacco (Manil, 11).  
Petunia mosaic in Japan (Matsumoto, 12).

b. *Potato-latent-mosaic group:*

Potato mottle (ordinary latent mosaic or healthy potato virus).  
European X-virus.  
Johnson's potato ring spot.  
Spot necrosis (1).  
Attenuated spot necrosis (1).  
Potato D-virus (16).  
Hyoseyamus IV virus (9).  
British Queen streak.  
Masked latent mosaic (symptomless on tobacco, 8).

c. *Potato-veinbanding group:*

Typical veinbanding virus of potato.  
European Y-virus.  
Cucumber mosaic.  
Price's yellow and green cucumber-mosaic isolates Nos. 2, 6, 8.  
Valleau's Delphinium virus 10729.  
Stipple streak of potato.  
[Rugose mosaic of potato = a strain of veinbanding + a strain of latent mosaic].

d. *Potato-aucuba-mosaic group*:

Potato aucuba mosaic.

Canada streak of potato.

e. *Etch group*:

Etch.

Severe etch.

Blakeslee's Z-mosaic of *Datura*.f. *Tobacco-ring-spot group*:

Wingard's tobacco ring spot.

Yellow tobacco ring spot.

Price's tobacco-ring-spot isolates: "green ring spot," "ring spot No. 2."

g. *Pea-mosaic group*:

Osborn's pea mosaic No. 2.

Osborn's pea mosaic No. 3.

h. *Mild mosaic of potato*:

Typical mild mosaic (reaction weak, often indefinite).

Verplancke (18) has reported positive precipitin tests with serum and extracts of mosaic beet plants. His serum was negative towards extracts of beets with yellows, of mosaic dahlia, of potato with mottle, crinkle mosaic, mild mosaic, streak, streak mosaic, and leaf roll, of mosaic pelargonium or of monstera with anthurium mosaic. Verplancke's experimental procedures differed from the customary serological methods in a number of respects, and his findings were not confirmed by Gratia and Manil (10). The beet-mosaic reaction, accordingly, requires further study before including it in the list of specific plant-virus reactions.

By the use of special techniques (5), certain of the strains of the tobacco-mosaic group may be differentiated from one another, and the same may be said for certain strains of the latent-potato-mosaic group. With ordinary precipitin techniques, however, the viruses included in any one group are indistinguishable from one another, but no virus from any given group cross-reacts with viruses from any other group. From the serological point of view, all of the viruses included in any group behave as strains of the same virus type. In general, this classification agrees with the results obtained by a number of workers using infection tests, physical and chemical properties of the viruses, or acquired immunity tests as criteria of relationship.

*Nonreactive Viruses.* Juices containing the viruses of the following diseases have been tested for precipitin reactions. The techniques used were

similar to those that gave positive results with the viruses enumerated above. In no case, however, was a virus-specific precipitin reaction obtained.

<sup>1</sup>\*Aster yellows.

Peach yellows.

Potato witches' broom.

Potato leaf roll.

Potato mild mosaic, J-strain (Schultz).

Potato mild mosaic, V-strain (Schultz).

Potato mild interveinal mosaic (supermild mosaic).

Potato mild circular mottle.

\*Potato yellow dwarf.

Potato calico mosaic.

Potato spindle tuber.

Potato crinkle mosaic (crinkle of Schultz and Folsom, not crinkle of Murphy and McKay, nor of Quanjér).

Pea mosaic No. 1 (Osborn).

Bean mosaic.

Sugar-cane mosaic.

Sugar-beet curly top. (?)

Sugar-beet mosaic.

\*Tomato spotted wilt.

\*Celery mosaic } See discussion above.

\*Lily mosaic }

\*Crucifer mosaic.

The viruses that have failed to give specific precipitin tests in nearly all cases differ from those that yield precipitins in most or all of the following respects:

<i>Serologically active viruses</i>	<i>Serologically inactive viruses</i>
a. Readily transmissible mechanically, often at dilutions of 1:1000 or higher.	Mechanical transmissibility poor or lacking.
b. Relatively stable <i>in vitro</i> .	Relatively unstable <i>in vitro</i> ; usually destroyed by aging 2 days or less at room temperature.
c. Thermal inactivation point relatively high, usually stable at 55° C. or higher.	Thermal inactivation point usually below 55° C.
d. Readily systemic.	Sometimes very poorly systemic.

Several possible explanations may be suggested as reasons why some viruses yield juices that react serologically, while other viruses fail to do so. Thus, failure to demonstrate seric reactions with the viruses listed above may be due to insufficient virus antigen in the juice, to antigenic inactivity of the virus juice, to instability of the virus antigen *in vitro*, or in the experi-

<sup>1</sup> Viruses marked with an asterisk were tested both by the standard precipitin technique and the field method. The other viruses were tested only by the standard method and might possibly yield specific reactions by the field method.

mental animals, or to a combination of these factors. It is unlikely, but not impossible, that failure of a virus juice to react might in some cases be due to the fact that the supposedly healthy host juice, used for absorbing the sera, contains a masked strain of the virus in question, incapable of causing recognizable symptoms on the plant but capable of precipitating the antibodies specific for the virus in question. Observations indicate that the explanation differs with different viruses. For example, using frozen juices in precipitin testing, tobacco ring spot gives a much stronger reaction with its proper serum than do the etch viruses. On the other hand, when these 2 viruses are tested by the field method, the etches are much more active than tobacco ring spot. According to results with the ultracentrifuge (17), the etches have much more heavy-weight virus protein than tobacco ring spot, and all available evidence indicates that it is the heavy-weight virus proteins that produce the virus precipitin reactions. It is, therefore, apparent that, in the case of the etch group, poor tests after freezing the juices are due to the instability of the virus-antigen, present in abundance in fresh juice; on the other hand, the weaker field test with tobacco ring spot appears to be due to the lower concentration of virus-antigen in juices containing this virus.

Moreover, the field method demonstrates precipitin reactions with a number of viruses that fail to react if the juices are frozen (cucumber-mosaic strains, etch strains). In these cases it is evident that a failure to obtain reactions with the frozen juices is due to instability of the virus-antigens and not to their being present in insufficient amount. In the case of tomato spotted wilt, no precipitin reaction was obtained, nor indeed was one expected, since this virus is inactivated by heating for 10 min. at the temperature of rabbit's blood; hence any virus inoculated into a rabbit probably would be destroyed long before antibody formation could occur. A few of the very slow-moving or poorly systemic strains of tobacco mosaic fail to give good precipitin tests. In such cases the concentration of virus antigen is evidently the important factor, since such viruses are poorly transmissible mechanically, although Jensen's tests show that they are highly resistant to heat and to ageing *in vitro*.

The correlation that appears to exist between seric activity and virus resistance suggests that the viruses themselves are directly responsible for the precipitin tests; however, the possibility that the reactions are due to specific by-products of virus activity is by no means excluded.

*The Limitations of Plant-virus Serology.* It follows from the data presented that the serological approach to the problem of plant-virus classification is limited by the fact that many viruses fail to produce specific precipitin reactions. At the 28th annual meeting of The American Phytopathological Society, the suggestion was advanced (6) that the list of viruses amenable to serological investigation might be lengthened by the development of more sensitive techniques. The development of the field method for



virus testing, shortly afterwards, showed that such was the case, since it added to the list of serologically active diseases a number of etch and cucumber-mosaic strains. It is very possible that further developments in the preservation and concentration of viruses, or perhaps the use of cold-blooded animals in serum preparation, may enlarge the field of virus serology.

Even with the limitations of the method as they now exist, however, the viruses that do yield specific reactions are those that present the most difficult problems in determination on account of their widespread occurrence and extensive host ranges. The poorly transmissible viruses are in general well characterized by their vector relationships, where these are known, and they do not present a problem in identification and classification so great as that encountered in the case of the serologically active viruses. From this point of view, even with its limitations, the serological method affords an aid in classification of those viruses with which such an aid is most needed.

#### PART II. TECHNIQUE AND APPLICATIONS OF THE FIELD METHOD FOR IDENTIFYING VIRUSES

An account recently has been published of a method for identifying viruses in the field, which appears to represent a specific agglutination of the suspended plastids of freshly expressed virus-containing juices (7). Since the publication of this account, the field method has been subjected to further study with the following results.

*Objectivity.* In an endeavor to test the field method under conditions that would rule out any personal factor, a number of experiments were devised in which the viruses to be tested were unknown to the operator. A representative experiment will be described. Of 16 plants of Turkish tobacco, 10 had been inoculated with a strain of latent mosaic that, according to numerous observers, is completely symptomless on tobacco, although it causes a striking necrotic disease of pepper. At the time of the experiment, the 16 plants were indistinguishable. The plants were given to a colleague who rearranged them arbitrarily on a greenhouse bench and assigned arbitrary numbers to them. The 16 numbered plants were next turned over to an assistant who made inoculations from each plant to 2 pepper seedlings. They were then put in the hands of a second assistant who was given a supply of latent-mosaic serum and instructed to perform field-method precipitin tests on each plant. The results of the precipitin experiment, which were available at once, showed that 9 of the plants contained specific virus antigen. Two weeks later, when the pepper seedlings showed infection, it was found that every plant that had yielded a positive precipitin reaction also yielded inoculum infectious to the pepper seedlings, while the serologically inactive plants yielded no material infectious to pepper. One of the 10 inoculated tobacco plants evidently failed to become infected. This was shown by its

negative precipitin reaction and by the negative pepper tests. The identity of the inoculated tobacco plants was not known to any of the persons involved in the experiment until after the experimental results were all recorded.

Analogous experiments, performed with a number of other viruses, have been consistent in showing that the method is accurate with unknown viruses in the hands of relatively untrained operators.

*Laboratory Use of the Field Method.* The suggestion has been made (7) that the advantages of the field method might warrant its use in the laboratory as the basic method for precipitin testing of viruses. The experience of the past few months has shown that such is the case, and, in the writer's hands, the field method has almost completely replaced the older method for routine precipitin testing of virus juices. The advantages are its rapidity (1 hour as compared with 2 days, for juice preparations by the older method) and its greater sensitivity towards most viruses. Tobacco-ring-spot virus alone proved to be more sensitive to the frozen-juice method, as recounted above. In the laboratory the field method may be somewhat improved, as regards sensitivity, by centrifuging the freshly expressed juices as a sole preliminary step to testing. This reduces the amount of suspended material in the juices and, hence, makes a small precipitation more readily apparent. It also serves to precipitate the larger suspended fragments of the juices and, hence, reduces disturbance of the reactions due to spontaneous settling in the juices.

*Artifact Reactions.* Occasionally, a plant juice will autoprecipitate when alone or in the presence of normal or heterologous serum. Such artifact precipitations do not lead to false conclusions as to the identity of viruses, because their presence in control tubes causes all reactions with an autoprecipitating juice to be excluded from the experiment. Artifacts of this type are no more frequent with the field method than with the frozen-juice method. They are particularly frequent in the juices of over-mature, yellowing plants. Such juices show strong oxidation, but attempts to eliminate the artifacts by the addition of reducing agents were unsuccessful, as, also, were attempts to remove them by change in pH or salt concentration of the juices. Often 20 to 30 per cent of precipitin tests must be discarded for this reason, and it is to be hoped that further investigation on the metabolism of dying plants will lead to a practical method for eliminating such autoprecipitations.

*Preservation of Sera.* Since refrigeration is impractical in the field, experiments were performed in order to test various methods of preservation of sera at room temperature. Such experiments showed that treatment of the sera with  $\text{HgCl}_2$  at 1:4000 (addition of 1 part  $\text{HgCl}_2$  at 1:400 to 9 parts of serum) provided sera that, after 35 days at room temperature, were still sparkling clear and showed no measurable loss in titer. Nearly the same results were obtained by adding 1 part of 5 per cent phenol to 9 parts of

serum. Unpreserved sera, kept at room temperature for 35 days, in some cases lost all their precipitating power and were badly contaminated with bacteria.

*Optimal Amounts of Serum and Virus.* The relative proportions of serum and virus, as given in the original account of the field method, have continued to prove most satisfactory, except that the tobacco-ring-spot reaction was slightly improved by a change from 2 parts virus juice + 3 parts 1:9 serum to 4 parts virus juice + 1 part 1:3 serum.

*Future Applications of the Field Method.* The field method of testing for viruses is now restricted to the few laboratories where sera can be readily prepared. It has been shown, however, that the test is usable in the hands of untrained operators, that the sera may be preserved for long periods at room temperature, and hence may readily be shipped, and that the cost of preparing sera is relatively low if large animals are used. From these facts it follows that the use of the field method might be easily extended to a widespread application through the preparation and distribution of virus-immune sera by a central supply organization. The most important responsibility of such a supply source would be maintenance of virus stocks in a pure condition. Such a use of sera would make it possible to test geographically localized viruses without the dangers involved in shipping the viruses themselves, and would make the precipitin method accessible to investigators and teachers on a scale now impossible.

The agglutination of suspended plastids, as seen in the virus reactions, also is apparent if the juices of normal plants are tested against nonabsorbed antinormal-plant sera. From this fact it seems probable that the field method might prove to be a useful adjunct to or substitute for the present methods of classifying plants by blood reactions. It is possible that the field method might lack the precision of the techniques used by Mez and others in plant sero-systematics, but the ease and rapidity of performance warrant a testing of the field method in this connection. The field method as used with the plant viruses, coupled with a dialysis of all juices (to remove carbohydrates and other artifact-producing non-proteins), might serve as a desirable addition to the techniques of sero-diagnosis.

#### SUMMARY

Recent precipitin tests indicate that: Canada streak of potato is a strain of aucuba mosaic of potato; Blakeslee's Z-mosaic of *Datura* is a strain of the etch group; Price's cucumber-mosaic isolates are strains of the cucumber-mosaic group, although celery-mosaic, lily-mosaic, and Doolittle's cucumber-mosaic juices failed to react with sera for Price's cucumber mosaic; the European Y-virus of potato is serologically indistinguishable from the American potato-veinbanding virus; potato stipple streak also is of the veinbanding group, and, since, in the field, it is customarily associated with latent mosaic,

it is considered to be a type of rugose mosaic. Tobacco-mosaic virus, propagated in root-tissue cultures and in locally necrotic lesions, yields specific virus antigen. All of the viruses that have thus far proved serologically active are grouped according to their relationship reactions. A list is given of the viruses that have failed to give reactions. Possible explanations for this failure are discussed.

The field method of precipitin testing for viruses gave dependable results in the hands of unskilled workers to whom the identity of the virus materials was unknown. Data and suggestions are given regarding the use of the field method as a laboratory procedure, the elimination of artifact reactions, the preservation of sera, and the applications of the field method of precipitin testing.

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# SCLEROTIUM BATATICA TAUBENHAUS, A COMMON PATHOGEN OF RED CLOVER ROOTS IN KENTUCKY<sup>1</sup>

LAWRENCE HENSON AND W. D. VALLEAU<sup>2</sup>

(Accepted for publication June 30, 1937)

Fergus and Valleau (6) described blackening of red-clover root systems associated with clover failure on 3 soil experiment fields in Kentucky, but did not determine the cause. An investigation of this condition was begun by the writers in 1931, and a fungus, mildly pathogenic and capable of blackening red clover roots, was repeatedly isolated. It has been identified as *Sclerotium bataticola* Taub. The purpose of this paper is to report this fungus as a common pathogen of red clover roots in Kentucky, and give reasons why the name, *S. bataticola* Taub. (15), is retained for the sclerotial form in preference to the more commonly accepted *Rhizoctonia bataticola* (Taub.) Butler.

## MATERIAL AND METHODS

Diseased roots used in these studies were taken from several varieties of red clover, ranging in age from 2 months to 2 years, from the Berea and Lexington Soil Experiment Fields. Red-clover seeds, previously treated in water at 49° C. for 10 minutes, were placed in a 1:64 solution of HgCl<sub>2</sub> for the same length of time. They were then removed, washed in sterile distilled water, and planted on sterile moist filter paper. Small pieces of red-clover roots, each including diseased and healthy tissue, were added to the plates after treatment in 1:1000 HgCl<sub>2</sub> for 10 seconds, followed by 6 washes in sterile distilled water. Seedlings, as they became diseased, were removed aseptically and planted on acid agar. Although several different fungi were obtained from each dish, *Sclerotium bataticola* was obtained 55 times from 112 plates. The fungus also was isolated 95 times from 200 plates when small pieces of the diseased roots were placed on acid agar after being treated with bichloride of mercury, as mentioned above. Sclerotia from the crowns of dead plants, also, were used in obtaining pure cultures of the fungus.

## IDENTITY OF THE FUNGUS

Several strains of the fungus isolated from red-clover roots, while differing from one another, all appeared to fit the general description of this fungus as given by other workers (1, 5, 14, 15). The hyphae of young cultures are colorless, abundantly branched, with branches usually arising almost at

<sup>1</sup> The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

<sup>2</sup> The assistance of the Division of Forage Crops and Diseases, United States Department of Agriculture, is acknowledged.

right angles to the parent hyphae. The branches usually are constricted at the point of origin. No clamp connections were seen in these cultures. When the mycelium produced a sparse, resupinate, fan-like growth, sclerotia were formed within 36 hours; but when it produced an abundant aerial weft over the surface of the medium, which reached the dish cover, sclerotia were produced within 3 days. There were all gradations between these extremes. The sclerotia of the red-clover fungus were produced in large numbers on and in artificial media (Fig. 1). They are smooth, black, spherical, oblong,

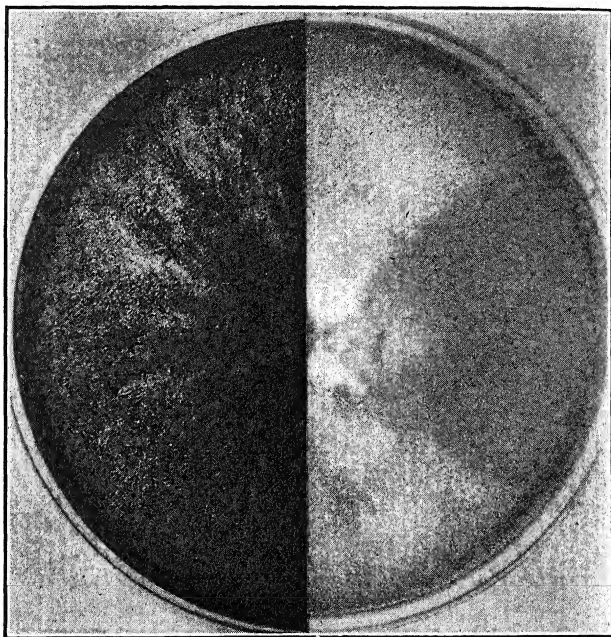


FIG. 1. Cultures of *Sclerotium bataticola* showing sclerotia, sectoring, and differences in strains when grown on 2 per cent potato-dextrose agar at laboratory temperatures.

oval, or curved bodies ranging in size from 61 to 150  $\mu$  on dead red-clover crowns, 57 to 145  $\mu$  on pepper fruits, and 70 to 242  $\mu$  on potato-dextrose agar. These measurements are within the size range reported by other workers. When crushed, the sclerotia are found to contain oil-like globules. Pycnidia were not observed by the writers on any one of a variety of media. Potato-dextrose (2 per cent) agar and potato plugs often are colored vinaceous to mineral red.<sup>3</sup>

Cultures of the organism obtained from California and North Carolina<sup>4</sup> were morphologically indistinguishable from the fungus obtained from red clover.

<sup>3</sup> Ridgway, R. Color standards and color nomenclature. Washington, D. C. 1912.

<sup>4</sup> These cultures were obtained from Dr. J. B. Kendrick and Mr. W. W. Mackie, and from Dr. R. F. Poole, respectively.

## PATHOGENICITY

In preliminary tests on over 1000 red-clover seedlings, growing in reduced light on filter paper moistened with potato broth, and on nutrient agar, the seedlings were killed by *Sclerotium bataticola* in from 4 to 10 days. The roots became black, but sclerotia were not observed on them.

Pure cultures of the fungus were added to soil, previously heated to 65° C., in which red-clover seed was planted. Many red-clover seedlings, soon after germination, were attacked by the fungus and were killed before reaching the surface of the soil; others died soon afterwards. Because of the loss of small roots the infected seedlings that lived developed much less extensive root systems than those in noninfested soil (Fig. 2). Only 1 black lesion was observed on the larger part of the taproot, from all the plants growing in infested soil (Fig. 2, A). *S. bataticola* was isolated from the cambium layer of this root about an inch below the visible lesion. After 50 days the soil was carefully washed from the roots and the total length of the root system of an average plant was measured. The root systems of the plants from the inoculated jars were from 1/7 to 1/2 the length of those from the noninoculated jars (Table 1).

TABLE 1.—Effect of *Sclerotium bataticola* on red-clover roots grown for 50 days (February 28 to April 19, 1933) in the greenhouse at Lexington, Kentucky

Variety	Inoculum <sup>a</sup>	Stand <sup>b</sup>	Length of root system in inches		Ratio of diseased to healthy roots	
			One-root system (measured)	Total root systems (calculated)	One-root system (measured)	Total root systems (calculated)
Ky. 101	Control	77	147	11,319	.....	.....
Ky. 101	41	39	85	3,315	1: 1.7	1: 3.4
Ky. 101	87	22	37	814	1: 4.0	1: 13.9
Tenn. 3	Control	73	223	16,279	.....	.....
Tenn. 3	41	60	31	1,860	1: 7.2	1: 8.8
Tenn. 3	87	36	115	4,140	1: 1.9	1: 3.9

<sup>a</sup> The number designates culture used. It was grown on agar and then mixed with soil. Controls received a proportional amount of sterile agar.

<sup>b</sup> Total number of plants grown in quadruplicate jars.

<sup>c</sup> Calculated root system is equal to the measured length of one root system multiplied by the total number of plants of stand.

## NOMENCLATURE

According to Ashby (1), Maublanc described a pycnidial strain of this fungus on beans in 1905 and named it *Macrophoma phaseoli*, n. sp. In 1912 Shaw (11) described a sclerotial disease on Jute, cowpea, etc., caused by this fungus and referred to it as *Rhizoctonia solani* Kühn. Taubenhaus (15), in 1913, described this organism from sweet potatoes and named it *Sclerotium*



*bataticola*, n. sp. In 1918 Butler (5) observed it on cowpea, groundnut, potato, tobacco, etc., and listed it as a *Rhizoctonia* largely on the basis of type of branching of the mycelium and on what he considered to be an occasional clamp connection. Shaw (12), in 1924, compared his cultures of *Rhizoctonia solani* from jute with cultures furnished by Taubenhause and concluded that they were morphologically indistinguishable and were not *R. solani*. Small (14), in 1924, independently placed this fungus in the genus *Rhizoctonia* on the basis of mycelial characters and on what he thought might be abortive basidia. He also saw what he took to be clamp connections, but his illustrations are not convincing. In 1925 Briton-Jones (2) submitted a cul-

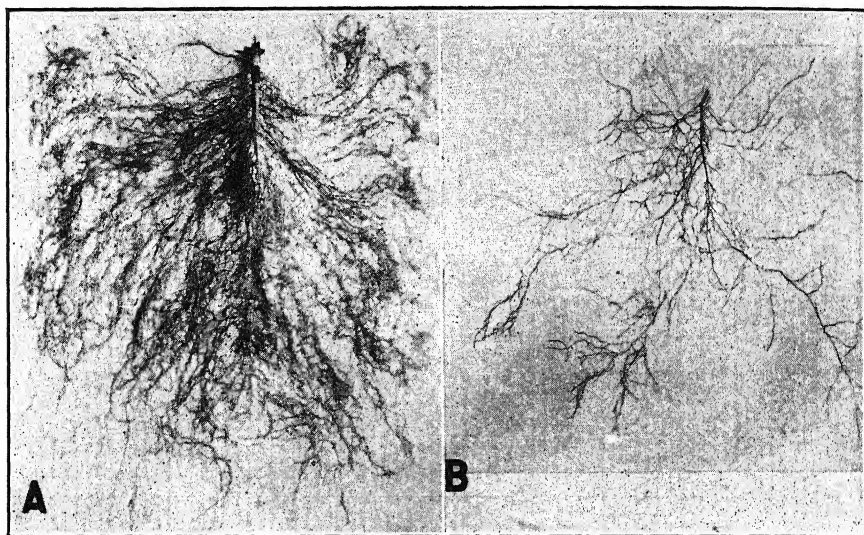


FIG. 2. Blackening produced on red-clover roots when they were grown in soil infested with *Sclerotium bataticola* in the greenhouse. A. Root system of plant seeded July 30, 1931. Photographed October 22, 1931. Note taproot with large black lesion, which is rarely produced by this organism. B. Lateral root from plant seeded and photographed as in A.

ture of the fungus from Egypt to Butler, who identified it as the *Rhizoctonia* sp. he had described from India, and with cultures received from Taubenhause from the United States. Briton-Jones then made the new combination, *Rhizoctonia bataticola*, based on Butler's earlier identification of his fungus as *Rhizoctonia* sp. In 1927, Ashby (1), working with a pycnidial strain of the fungus, made the new combination, *Macrophomina phaseoli* (Mauhl.). Haigh (7), and Hopkins (8) warned against the use of the name *M. phaseoli* for all the sclerotial forms that are now included in *R. bataticola*.

Rolfs (10) demonstrated that *Rhizoctonia solani* is the basidiomycete,



*Corticium vagum*; and Buddin and Wakefield (3, 4) showed that *Rhizoctonia crocorum* has, as its perfect form, *Helicobasidium purpureum*. Recently, Kotila (9) described a *Rhizoctonia* as *Corticium praticola*. It would seem, therefore, that the genus *Rhizoctonia* should be considered as the imperfect stage of a basidiomycete.

The fungus under discussion has not been proved to be a basidiomycete, but actually appears to have more of the characters of an ascomycete. Proof that it produces clamp connections is wanting. The type of branching, upon which it was evidently first identified as a *Rhizoctonia*, is not a reliable character for separation of basidiomycetes from ascomycetes. For example, *Sclerotinia sclerotiorum* was reported by Shaw and Ajrekar (13) as *Rhizoctonia napi* West; and *Sclerotium rolsii* was for a long time thought to be related to the Sclerotinias because of the similarity of mycelial characters, but is now known to be a basidiomycete. There seems to be ample evidence for the belief that *S. bataticola* sometimes produces pycnidia of the type commonly produced by ascomycetes. Pycnidia are extremely rare, if not entirely lacking, among the true basidiomycetes. It appears, therefore, that there is no basis at the present time for naming this fungus *Rhizoctonia*. It is, therefore, suggested that the sclerotial strains of this fungus be referred to as *Sclerotium bataticola* rather than *Rhizoctonia bataticola*.

#### SUMMARY

*Sclerotium bataticola* Taub. was isolated from blackened rootlets, taproots, and crowns of red clover. It is mildly pathogenic and is capable of causing a seedling blight and blackening of red-clover roots when grown in the greenhouse.

The name *Sclerotium bataticola* Taub. is synonymous with *Rhizoctonia bataticola* (Taub.) Butler; but, inasmuch as *Rhizoctonia* has frequently been shown to be the imperfect form of a basidiomycete, and as the fungus under consideration has not been shown to have any characters typical only of the basidiomycetes, it seems preferable to use the name *Sclerotium bataticola* for the sclerotial strains of the fungus.

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## CARBON AND OXYGEN REQUIREMENTS OF THE COTTON ROOT-ROT ORGANISM, *PHYMATOTRICHUM* *OMNIVORUM*, IN CULTURE<sup>1</sup>

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### INTRODUCTION

Because of the economic importance of cotton root rot in the Southwest, the physiology of the causal organism, *Phymatotrichum omnivorum* (Shear) Duggar, is of great significance, the specific name indicating the wide variety of hosts attacked.

This fungus is not exacting in nutritional requirements (2). A wide assortment of carbohydrate and nitrogen sources were found to support satisfactory growth accompanied by the production of acid. The growth of the root-rot fungus is checked by anaerobic conditions and restricted by concentrations of carbon dioxide greater than 25 per cent (3), but is not killed by prolonged exposure to anaerobic conditions.

Wilson (6) reports a comparison of growth and acid production by *Phytomonas rhizogenes* Riker et al. and *Phytomonas tumefaciens* (S. and T.) Duggar on yeast infusion medium with 1 per cent glucose in both liquid and agar. He found that these organisms decompose most of the glucose in the agar within 10 days, while the growth in liquid is much slower and the glucose not all consumed in 30 days. The change in the pH of the medium is consistently more rapid in agar than in the corresponding liquid medium.

<sup>1</sup> Contribution from The Clayton Foundation.

The persistence of *Phymatotrichum omnivorum* in the soil is well-known (4). Further knowledge of its carbon nutrition may be expected to furnish information as to whether the compounds found in the soil are such as to permit active growth in the absence of living tissue. Although the host range of the fungus is large, it is sharply limited by the virtual immunity of the Monocots under field conditions of Central Texas (5). More extensive knowledge of the carbon nutrition may be expected to be useful in understanding this phenomenon. Finally, any acids produced may be expected to affect the fungus or host and to be of importance in the relations existing between the parasite and the other microorganisms with which it is associated.

#### METHODS

This study was made by using pure cultures of *Phymatotrichum omnivorum* and by growing the fungus in liquid and on agar media containing the same mineral nutrients and a single carbohydrate in 1 per cent concentration. The stock medium was made according to Koser's basic formula, which contains: Sodium chloride 5.0 g., magnesium sulphate 0.1 g., monammonium phosphate 1.0 g., potassium monohydrogen phosphate 1.0 g., 1 per cent brom cresol purple 1 cc., and distilled water 900 cc. All carbohydrates were made in 10 per cent concentrations, autoclaved separately from the basic medium, and added to it under sterile conditions to give a concentration of carbohydrate of 1 per cent; thus diluting the basic formula 9 to 10. These media were divided into 25 cc. amounts in large tubes; half of these were made solid by the addition of 2 per cent agar and the remainder left liquid. The pH in all cases was adjusted to neutrality, using as indicator brom cresol purple. All media were incubated several days prior to inoculation in order to insure sterility. Inoculations were made from actively growing 10-day cultures of *P. omnivorum* on potato dextrose medium. The size of the inoculum was standardized to  $5 \times 5 \times 2$  mm.

The utilization of the carbohydrate, as indicated by the acidification of the medium, is shown by the color change of the entire culture from wine red to yellow, corresponding to pH 6.8 and 5.2 and, also, as shown by the comparative estimated recorded growth of the fungus. A scant growth occurred in the medium that lacked a carbohydrate except for the small quantity introduced with the inoculum.

Comparative estimates were made of growth and of the time necessary for acidification, using, as a carbon source, dextrose, levulose, galactose, maltose, sucrose, lactose, mannite, xylose, inulin, dextrin, soluble starch, potato starch, corn starch, glycerin, and cellulose. Determinations were made on media in which the carbohydrate supply was root decoctions. These decoctions were made by steeping for 1 hr. 10 g. dry root material in 100 cc. distilled water, filtering, autoclaving and adding to the basic formula in the

proportion of 1 to 9. The roots of cotton, *Gossypium herbaceum* L., and Johnson grass, *Holcus halepensis* (L.) Pers., were used. Controls of Koser's base were included in all cases. Experiments were carried out at room temperature, 20°–25° C., in diffuse light. Observations were made at daily intervals and recorded each week, or oftener, if the results justified a record.

Since the free atmosphere and that of the soil frequently differ in oxygen content, and in view of the report of Neal and Wester (3) on the dilution effects of carbon dioxide, cultures were set up with atmospheres of various oxygen concentrations. Cultures were made in both liquid and agar media with the various sources of carbohydrate, as detailed above, and allowed to grow for 48 hours before subjecting them to the various concentrations of oxygen. The culture tubes were arranged against the sides of large museum jars so that observations could be made without opening. In one set, room atmosphere was sealed by means of Plasticene. In the second set, one-half of the oxygen was removed, following the calculations of Ehrenberg (1) by the pyrogallie acid method, thus establishing an atmosphere of approximately 10.5 per cent oxygen. In the third set of jars, anaerobic conditions were established by the phosphorous method. For the fourth set, the pressure was reduced in Novy jars so that, when brought back to normal by the introduction of oxygen from a cylinder, an atmosphere of approximately 42 per cent oxygen was established. All jars were incubated at room temperature and observations were made at intervals for 6 weeks.

Cultures growing on the agar media were predominantly surface and, so, were exposed to the varied experimental conditions. Those growing in the liquid media were largely submerged and, hence, grew under conditions affected by the fungus itself and only indirectly by the gases with their different solubilities and diffusion rates. The subsurface of liquid cultures in normal atmosphere rapidly approaches anaerobic conditions (4).

#### EXPERIMENTAL RESULTS

In a preliminary experiment two isolations of *Phymatotrichum* were used in order to determine any differences in their ability to utilize different nutrients. Isolation No. 24, secured from Dr. Taubenhau, had been carried in laboratory culture for several years with no apparent decrease in vigor of growth; isolation No. 1 was recently cultured in this laboratory from a field sclerotium. The carbon compounds used in both liquid and solid media included hexoses, pentoses, and their polymeres, glycerin, and root decoctions that included the water-soluble substances occurring in the tissues.

Variable growth occurred in all cases in both liquid and solid media. No growth occurred in the controls in which no carbon nutrients were placed. Acidification was much less consistent. Acidification occurred on the agar cultures during the course of the observation period in all cases except corn

starch and the cotton-root decoction (Johnson grass-root decoction was not included in this experiment) with both fungus isolations, and, in the case of cellulose, with isolation No. 24. Acidification occurred on agar more frequently and in less time than in liquid for both isolations. The average time required for reaching a pH of 5.2 was 11 days on agar and 14 days in liquid for all cases of acidification. Although the results with the 2 isolations vary, no significant differences appear. To simplify further experiments, isolation No. 1 was used. This preliminary work shows that the root-rot fungus can utilize a considerable range of carbon compounds for growth, and that, while growth may vary in vigor, prolonged cultivation on synthetic media does not materially change the availability of different compounds.

In the next group of experiments tubes of both liquid and agar media were inoculated in quadruplicate with isolation No. 1 and allowed to incubate at room conditions for a period of 5 weeks. Carbon sources were similar to those given in the preliminary experiment, except that mannite was omitted and potato starch and Johnson grass-root decoction were added. Since it was felt that the oxygen supply might account for the differences observed between the liquid and agar cultures, a note was made of those liquid cultures in which the growing fungus colony floated at the surface, where conditions might be considered comparable to those prevailing at the surface of agar colonies. The average number of days required for acidification of the medium and the average amount of growth that occurred are given for each type of medium in columns under "Room conditions" in table 1.

The results recorded in table 1 are averages from several experiments. Acidification occurred much more rapidly on the agar cultures than in the liquid, the average for all cultures showing acidification was 12 days for agar cultures and 27 days for the liquid. The results are similar to those obtained in the preliminary experiments except that a somewhat longer period was necessary for the liquid cultures to become acidified. In no case, in either liquid or agar, did acidification occur in the media containing the root decoctions. Considering both liquid and agar media, acidification was most rapid in levulose and xylose. Acidification was, however, more than 3 times as fast on agar as in liquid, when dextrose, maltose, sucrose, dextrin, or soluble starch were used as a carbon source. Acidification in floating cultures was not materially increased over those in which the colonies grew submerged; these individual differences are not included in the averages given in table 1.

Results show that, as a rule, a fair to good growth occurred in all cases. On the agar media the poorest growth occurred in glycerin, root decoctions, and the controls. In general, the growth in liquid media did not equal that occurring on agar; floating colonies did not appear significantly better than

TABLE 1.—Average number of days necessary for acidification and comparative growth in various carbohydrate media at room conditions and at various oxygen concentrations by isolation No. 1 of *Phymatotrichum omnivorum*

Carbohydrate 1 per cent	Room conditions		21% oxygen		42% oxygen		10.5 oxygen	
	Agar	Liquid	Agar	Liquid	Agar	Liquid	Agar	Liquid
Dextrose .....	8.7g	30g	11vg	21p	7g	7g	11.5g	28p
Levulose .....	8.7g	8.7g	11vg	31.5g	15.6g	21.3g	13g	25p
Galactose .....	12g	31.5g	11vg	31.5g	17.5g	21g	21g	35g
Maltose .....	7vg	33f	15.6vg	21p	13g	28.5g	17g	21p
Sucrose .....	8.7vg	31.5g	11vg	21p	18g	30g	21.6g	p
Lactose .....	12.2g	35f	20g	28g	25g	30g	19g	p
Xylose .....	8.7g	7g	11vg	g	13g	14.5g	9.5g	p
Inulin .....	12g	22.7g	20vg	17.5g	16.6g	31.5g	19g	21p
Dextrin .....	8.7g	28g	13vg	21g	13g	31.5g	13g	28p
Sol. starch .....	7g	24.5g	13g	31.5g	16g	23g	13g	31.5p
Potato “ .....	19.2g	g	11vg	31.5g	18g	21.5p	15.6g	24.5p
Corn “ .....	17.5g	35g	15.6g	35p	14g	7p	14.5g	28p
Cellulose (filter paper) .....	28g	35f	12g	g	7g	p	22g	21p
Cellulose (cotton) .....			16g	p	15g	p	g	p
Glycerin .....	p	g	12p	42p	23g	p	16p	p
J. Grass root .....	f	f	g	g	g	g	p	p
Cotton root .....	f	f	p	g	g	g	p	p
Control .....	p	p	p	p	p	p	p	p
Averages .....	12	27	14.3	29	16.9	25.3	16.9	25.7

Legend: p—poor growth, f—fair growth, g—good growth, vg—very good growth.

the submerged ones. Since table 1 includes only averages, individual culture conditions are not shown. The least growth in liquid occurred in maltose, lactose, corn starch, and cellulose; the corresponding agar media exhibited good growth. Controls showed scant growth in all cases, indicating the utilization of the small amount of nutrient introduced with the inoculum or of the agar or substances associated with it. The growth on both root decoctions in liquid and in agar was fair.

The results indicate the wide range of carbohydrates utilized by the fungus and the usual acidification of the media, with the exception of the root decoctions, glycerin, and the controls. Agar cultures are more favorable than liquid for the acidification of maltose, dextrose, sucrose, dextrin, and soluble starch, but this relation did not hold for xylose and levulose. Decoctions of roots of susceptible cotton and immune Johnson grass showed no noticeable difference in the amount of fungus growth they supported in the concentrations used.

Studies with 12 duplicate cultures placed in atmospheres of different oxygen concentration were carried out. Since these cultures were checked by the simultaneous cultures at atmospheric conditions and were carried out subsequently to those already discussed, the second section of table 1, gives under 21 per cent oxygen, the average results of the checks that are strictly comparable to the cultures placed under the different oxygen concentrations.

Under atmospheric conditions, 21 per cent oxygen, acidification occurred on liquid and agar media, although the time was variable with all nutrients except root decoctions and the controls. The average time required for acidification (where it occurred) was 14.3 days on agar as against 12 days required for the same acidification under room conditions. In the liquid media the acidification was considerably delayed, an average of 29 days being necessary for it to occur. This was not only a longer period than was required for acidification of the agar media, but was also slightly longer than the 27 days necessary under room conditions. No acidification occurred in the root decoctions nor in the controls.

Growth was accompanied by acidification of both liquid and agar media in all cases except the root decoctions and the controls; the rates of acidification and the amounts of growth produced showed no close parallel in the individual cultures.

Under conditions of twice normal atmospheric oxygen, 42 per cent, the frequency and the time of acidification did not differ significantly from that occurring in normal atmosphere. On the average, acidification, where observed, again occurred more rapidly on the agar cultures than in the corresponding liquid ones, the difference in time being slightly reduced from that observed at normal atmosphere. However, the difference in time required for acidification, where it occurred, between agar and liquid was

reduced. These differences between the average time for acidification for agar and liquid were 14.7 days at 21 per cent oxygen and 8.4 days at 42 per cent oxygen. Growth at 42 per cent oxygen was good, but not equal on agar to that at 21 per cent oxygen. Growth on the agar media was slightly better than in the corresponding liquid, but the difference was less than at 21 per cent oxygen. Root decoctions and the controls showed results similar to those obtained at 21 per cent oxygen.

If results obtained at decreased oxygen, 10.5 per cent, are compared to those obtained at 21 per cent, it is evident that in the former the time required for acidification was slightly increased in the agar, and the number of cultures showing this phenomenon during the experimental period decreased, particularly in the liquid. No significant differences from those already discussed appeared in the utilization of the carbon compounds as indicated by the acidification of the media; results were variable but showed uniformly no acidification in the root decoctions nor in the controls. Growth was slightly reduced in agar cultures, but was strikingly reduced in the liquid ones. Data that can not be included in the averages in table 1 showed the growth in liquid to be but 70 per cent of that occurring at 21 per cent oxygen.

Under the method used for obtaining anaerobic conditions, cultures were maintained at reduced pressure, which made perfect sealing difficult. In most cases neither acidification nor growth occurred. The results have thus not been included in table 1. In the few cases in which delayed acidification and poor growth occurred, it is possible that imperfect sealing may have been responsible.

The results given in table 1 indicate that the fungus can utilize a similarly wide range of carbon compounds under atmospheric conditions varying from 42 to 10.5 per cent oxygen. Activity is checked under anaerobic conditions. In general, agar media were more favorable than the corresponding liquid ones for growth and acid production at all oxygen concentrations that permitted activity. Acidification always is accompanied by growth, but growth without acidification may occur at all oxygen concentrations tried; this generally happens in the cellulose, glycerin, root decoctions, and the controls. Growth, particularly in liquid media, is reduced under conditions of 10.5 per cent oxygen. At high oxygen concentration the difference between the average time necessary for acidification (where it occurred) of agar and liquid cultures was reduced. At decreased oxygen concentration the averages neared those obtained at normal oxygen.

Obviously, the acidification of the medium by the growing fungus, with an available supply of oxygen, will require the production of an acid or the removal of a base in quantities depending on the buffer action of the medium. This was determined for 25-cc. samples of selected media such as were used in the culture work (Table 2).



TABLE 2.—*Number of cubic centimeters of N/100 HCl necessary to titrate 25-cc. samples of various culture media from pH 6.8 to 5.2*

Medium	Average cc.
Koser's .....	4.0
Koser's plus 1 per cent dextrose and 2 per cent agar .....	8.8
Koser's plus 1 per cent dextrose .....	4.0
Koser's plus 1 per cent glycerin .....	4.0
Koser's plus 1 per cent cotton-root decoction .....	4.05
Koser's plus 1 per cent Johnson grass-root decoction .....	4.06

It is obvious that the agar acts to a considerable degree as a buffer, yet it is on this medium that acidification takes place most rapidly. If oxalic acid be assumed to be formed during the incomplete oxidation of dextrose, computations show that less than 0.2 per cent of the sugar present in the culture is sufficient for acidification of Koser's medium with or without 1 per cent dextrose. The fact that 6 days or more were required for this production is in line with the fact that some growth was supported by the 50-mm. inoculum in which the original amount of dextrose was but 0.05 mg. The metabolic requirements of the fungus are low, and since, even with 42 per cent oxygen, acid is formed and incomplete oxidation of the carbohydrate indicated, the oxidative activities are probably low.

Since the availability of oxygen shows a general relation to growth and acidification, and only the surface layers of a culture are directly exposed and the subsurface supply depends on the solubility, concentration, and diffusion, it is obvious that aeration conditions on agar slants are very different from those in liquid cultures. In order to approximate more closely similar physical conditions in the cultures, the next series of experiments was carried out with 25-cc. amounts of the media placed in 250-cc. Ehrlenmeyer flasks. This gave equal surface exposure to the atmosphere and equal depth of the medium—7 mm. in both agar and liquid. Comparative cultures were placed in tubes similar to those used in the preceding work; a second series of tubes of liquid cultures was inoculated so as to float the inoculum on the surface film (Table 3).

Acidification uniformly occurred in agar media in flasks more quickly than in agar slants, the averages being 12 and 14 days, respectively. Although potato starch, corn starch, and cellulose developed acid only in the flasks, this is not significant, since these media had previously developed acid in the tube cultures, as shown in table 1. The liquid cultures in which the colonies floated at the surface are placed in separate columns in table 3 from those in which the colonies grew submerged. The rate of acidification in liquid cultures is variable, but acidification occurred in only a few cases in which the fungus produced a submerged colony. The significance of the average number of days necessary for acidification, namely, sub-

TABLE 3.—Number of days necessary for acidification and comparative growth on various carbohydrate media in flasks and tube cultures at atmospheric conditions for isolation No. 1 of *Phymatotrichum omnivorum*

Carbohydrate 1 per cent	Tubes of 25 cc. each			Flasks of 25 cc. each		
	Agar	Liquid		Agar	Liquid	
Dextrose .....	12g	p	12g°	10g		17g°
Levulose .....	12g	23g		10g		17g°
Maltose .....	12g		12g°	10vg	p	
Sucrose .....	12g	p	12g°	10vg		17g°
Lactose .....	12g	21p		10vg	p	
Xylose .....	12vg		12g°	10vg		17g°
Inulin .....	12g	21g	5g°	10vg		10vg°
Dextrin .....	12g	34p	5g°	10vg		10vg°
Soluble starch .....	12g	34g	12g°	10vg		10g°
Potato " .....		p		10vg		10g°
Corn " .....		g	5g°	10vg		17g°
Cellulose (filter paper) .....		12g		17g	28p	
Cellulose (cotton) .....	34g	35p		28g	28g	
Glycerin .....	12g	g		17vg	p	
J. Grass root .....	g	g		p	p	
Cotton root .....	g	f		g	g	
Control .....	p	p		p	p	
Averages .....	14	25.7	9.3	12	28	13.8

Legend: p—poor growth, f—fair growth, g—good growth, vg—very good growth.  
° Colony floating at surface.

merged tube cultures, 26 days; floating tube cultures, 9 days; submerged flask cultures, 28 days; floating flask cultures, 14 days; is limited. Nevertheless, the results justify the conclusion that the rates of acidification are greater in floating than in submerged colonies in both tubes and flasks. This is evident from an inspection of table 3, which shows that acidification developed during the course of the experiment in all floating cultures, but failed to develop in 14 of those that were submerged. In these cases the times of acidification, being undetermined, do not appear in the averages. There is no significant difference between the cultures submerged in 7 mm. media and those in the tubes, several centimeters from the surface.

The data for 5 carbohydrates, dextrose, sucrose, inulin, dextrin, and soluble starch, may be used as a criterion for the 3 culture conditions, agar, submerged in liquid, and floating in liquid. The average acidification time for agar cultures in these media, both in flasks and tubes, and for all floating liquid cultures is the same—11 days; while, for all submerged cultures, it is 29 days. Since the results in table 3 do not include the instances in which acidification did not occur during the experimental period, they do not completely represent the case, for only in submerged cultures did acidification fail to develop. Aeration conditions are effective in the rate at which a medium becomes acidified and in the extent of growth the fungus produces.

Better growth generally occurred on the agar media in flasks than on the agar tube slants. The best growth in liquid developed in the flasks and the

poorest in the tubes in which colonies grew submerged. Floating tube cultures showed an intermediate but good growth.

In general, table 3 indicates that those culture conditions that facilitate the absorption of oxygen favor rapid acidification of the medium and a high growth rate by the fungus.

#### DISCUSSION

It is obvious that the atmospheric conditions under which the root-rot fungus is grown profoundly affect its activities. It is also evident that these effects may be altered by the solid or liquid condition of the medium in which it is grown and by the position which the fungus colony occupies. If, in the preceding work, the averages of which are given in tables 1 and 3, the relative growth is evaluated as:  $p = 1$ ,  $f = 2$ ,  $g = 3$ ,  $vg = 4$  and this value multiplied by 100 to avoid fractions, we may secure a utilization quotient, representing the comparative availability of the nutrients, by using the number of days required for acidification as the divisor. Table 4 records these utilization quotients and the comparative availability of the carbon compounds; the letters A, B, and C indicate those carbohydrates that are most favorable for acidification and growth, the next most favorable, and the least favorable under a given set of conditions.

It is evident from table 4 that, with all oxygen concentrations tried, dextrose, maltose, dextrin, soluble starch, and xylose are the most readily available of the carbohydrates in agar; that is, their utilization quotients are the highest. In liquid cultures, under all oxygen concentrations tried, dextrose, inulin, dextrin, xylose, and soluble starch have the highest utilization quotient averages. Xylose is one of the most readily available carbon compounds tried, considering all culture conditions. Levulose seems particularly favorable under conditions of low oxygen concentration in submerged cultures. Least favorable of all the carbon compounds tested were cellulose (filter paper and cotton) and glycerin under all oxygen concentrations tried. The other carbon compounds fall in an intermediate group according to their average utilization quotients.

The utilization quotients average much less for liquid than for agar cultures at the oxygen concentrations tried. Averages for values given in table 4 of the submerged liquid cultures are 3.5, 8.5, and 12 for 10.5, 21, and 42 per cent oxygen concentrations. The corresponding values for the agar cultures are 16.5, 26.6, and 20.9. Evidently, atmospheric concentration of oxygen is near the optimum for the fungus, and 42 per cent is beyond, when the mycelia are directly exposed on the agar. Cultures floating in liquid at 21 per cent oxygen give the average value of 28.2 as utilization quotient, thus approaching the value obtained on agar at the same oxygen tension. It is probable that a concentration greater than 42 per cent could be found

TABLE 4.—*Favorability of various carbohydrate media at different oxygen concentrations for acidification and for growth of Phyto-*  
*motrichum omnivorum*

Carbohydrate	21 per cent oxygen			42 per cent oxygen		10.5 per cent oxygen	
	Agar	Liquid submerged	Liquid floating	Agar	Liquid submerged	Agar	Liquid submerged
Dextrose	B 30.7	C 5.4	B 20.6	A 42.8	A 42.8	A 26	A 3.5
Levulose	B 30.7	A 13.9	B 17.6	B 12.8	A 14	A 23	A 4
Galactose	B 30.4	B 9.5		C 11.4	A 14.2	B 14.2	B 2.8
Maltose	A 33.7	C 4.4	A 25	A 23	B 10.5	A 17.6	A 4.7
Sucrose	B 30.7	C 5.7	B 20.6	B 16.6	B 10	B 13.8	B 2.8
Lactose	B 24	B 5.9		C 10.7	B 10	B 15.7	B 2.8
Xylose	A 36.5	A 14.2	B 20.6	A 23	A 20.6	B 15.7	A 4.7
Inulin	B 25.9	A 14.7	A 46.6	B 18	B 9.5	B 15.7	A 3.5
Dextrin	A 32	B 8.8	A 46.6	A 23	B 9.5	A 23	A 3.1
Soluble Starch	B 30.9	A 10	A 27.2	B 18.7	B 13	A 23	A 4
Potato	A 33.3	B 8.8	A 30	B 16.6	B 4.6	B 12.8	A 3.5
Corn	C 23.2	B 6.6	A 27.2	A 21.4	A 14.2	A 20.6	A 4.7
Cellulose (Filter paper)	C 15.7	B 8.1		A 42.8	C 2.8	B 13.6	B 2.8
Cellulose (Cotton)	C 11.5	C 5		A 20	C 2.8	C 6.6	B 2.8
Glycerin	C 11.8	C 5.4		B 13	C 2.8	C 6.2	B 2.8

Legend: A—Those carbohydrates most favorable to acidification and growth.

B—Those carbohydrates next most favorable to acidification and growth.

C—Those carbohydrates least favorable to acidification and growth.

average growth  $\times 100$ .

Figures are average number of days required for acidification = U.Q.

Relative growth is evaluated: p-1, f-2, g-3, vg-4.

U.Q. Utilization quotient.

in which the utilization of the nutrients would reach the value shown by surface colonies on agar at 21 per cent oxygen.

It would seem from the results of this study that the presence of small amounts of minerals and carbohydrates in the soil is adequate for the growth of the root-rot fungus. Cellulose or the simpler carbohydrates may serve, providing adequate oxygen is available to the mycelia. Field studies, the results of which are not published, show that during midsummer the oxygen content of the soil atmosphere in the cotton fields may be reduced as low as 12 per cent in the deeper layers. The effect of this condition may be expected to be both a reduction in the growth of the fungus and a restriction of the number of favorable nutrients. Since, in the black lands of Central Texas, much of the carbon dioxide appears in the soil as bicarbonates, the pH is not greatly altered. Wind, rain, and cultivation result in aerating the superficial layers in which the attack on plants by the root-rot fungus probably occurs and in which growth conditions can be expected to be most favorable for the fungus.

There is no evidence that there are any nutrients more favorable in the susceptible cotton roots than in the resistant Johnson grass roots. The water-soluble nutrients of root tissues were not favorable media for the growth of the fungus in culture in the concentrations used in this work and never developed acid during the observation period under any conditions tried. The explanation of this probably lies in the low carbohydrate concentration present in the root decoctions (0.2 per cent) used in these experiments. No difference appeared at this low decoction concentration between cotton and Johnson grass.

It is obvious that culture studies of this fungus must be interpreted in the light of physical, as well as chemical, conditions. The variable behavior of many fungi in culture may be due to a failure to control physical conditions that affect the availability of oxygen. In the soil the surfaces of the mycelia are in part directly exposed to the atmosphere somewhat in the manner of the surface growth on agar. Culture studies approach conditions in the soil more nearly in agar than in liquid.

#### SUMMARY

*Phymatotrichum omnivorum* is capable of utilizing a great variety of carbon compounds as nutrients. Dextrose, levulose, galactose, maltose, sucrose, lactose, mannite, xylose, inulin, dextrin, soluble starch, potato starch, corn starch, glycerin, and cellulose were used.

Prolonged cultivation on synthetic culture media does not materially change the availability of the different carbon compounds.

Acidification of the media occurred rarely with cellulose (filter paper or cotton) and glycerin, and never occurred in the dilute root decoctions nor in the controls. Acidification occurred in all other carbohydrate media.

Growth may occur with or without acidification of the media. Agar cultures are somewhat more favorable for growth than liquid and show considerably more rapid acidification in spite of the fact that they are more highly buffered.

With normal atmosphere, the rates of growth and acidification by floating colonies in liquid culture approach those on agar, while the rates of submerged colonies are low and vary with the oxygen concentration.

Liquid and agar cultures, with the same surface and volume, show relations similar to those discussed for tube cultures.

Anaerobic conditions check the activities of the fungus.

Forty-two per cent oxygen increases the activity of the fungus in liquid cultures, but does not have this effect on agar cultures. The difference in time necessary for acidification in agar and liquid cultures is thus decreased.

Ten and one-half per cent oxygen decreases the activity of the fungus slightly on agar cultures and markedly reduces it in liquid.

If the growth of the fungus and the acidification of the medium are expressed as utilization quotients, the averages are much less for liquid than for agar cultures at the oxygen concentrations tried. When cultures are submerged the quotients vary directly with the oxygen concentration. In agar cultures the highest utilization quotient occurs in normal atmospheric oxygen. Oxygen at normal atmospheric concentration is near optimum for the fungus when the mycelia are directly exposed.

The metabolic activities of the root-rot fungus are closely associated with the oxygen supply to the mycelia. This may be affected by the oxygen partial pressure and the exposure of the mycelia.

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# SULPHUR AND ROSIN AS DOWNY MILDEW FUNGICIDES

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## REVIEW OF LITERATURE

Sulphur frequently has been tested as a fungicide for various downy mildews, but generally has been found of little value. Doran (3, 4), Newton and Yarwood (8), Newton and Hastings (7), Yarwood (9), Zeller (10), and investigators in Wales (1), have reported on the toxicity of rosin combinations to fungi.

## METHODS AND MATERIALS

In the present studies, Bordeaux mixture, lime sulphur, rosin, and mixtures of rosin and lime sulphur were tested as fungicides for the control of the downy mildew of onion (*Peronospora destructor* (Berk.) Caspary on *Allium cepa* L.) and the downy mildew of hop (*Pseudoperonospora humuli* Miyabe and Takahashi on *Humulus lupulus* L.). As neither Bordeaux nor lime sulphur spread well on onion and hop foliage, certain spreaders were added.

Bordeaux mixture was prepared with equal parts of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  and  $\text{CaO}$ , and the concentration of Bordeaux is stated as the concentration of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  in the dilute spray. The Penetrol spreader added to Bordeaux is a proprietary miscible oil.

Lime sulphur was prepared from commercial lime-sulphur solution (guaranteed not less than 29 per cent calcium polysulphides and having a measured specific gravity of 1.26). The dilutions of lime sulphur indicated are by volume. The S.O.S. spreader, used with lime sulphur, is a proprietary form of sodium oleyl sulphate.

The stock rosin was prepared by heating together 5 parts E grade rosin, 1 part KOH, and 14 parts of water. The stock rosin solution was diluted with water as required and the concentration of the spray solution is expressed as the percentage by weight of rosin in the diluted spray.

The rosin-lime-sulphur spray was prepared by adding concentrated lime sulphur to dilute rosin solution. A straw- or sulphur-color gelatinous precipitate, much superior in stability to Bordeaux precipitate, as measured by settling tests, was produced. This mixture is more viscous than Bordeaux per unit of dry matter in the dilute spray and spreads well on onion and hop foliage. It leaves a conspicuous deposit on foliage, somewhat similar in appearance to Bordeaux + Penetrol, and resembles Bordeaux in not being easily removed by rains.

Because of the uncertainty and unevenness of natural infection, artificial inoculation has been resorted to in most tests. Leaves or plants were inoculated by atomizing them with a concentrated spore suspension in the evening. They were then subjected to an adequate incubation in the moist chamber, in the greenhouse tests.

In testing the effect of spray materials on sporulation, naturally or artificially infected leaves in which the fungus was vegetatively mature, but had not sporulated, were sprayed with the fungicide, allowed to dry, and placed overnight in greenhouse moist chambers. The relative amount of sporulation was recorded the following morning.

In the tests of resistance of fungicides to weathering, greenhouse seedling onions of the Prizetaker variety, about 6 weeks old, were sprayed with the test fungicide, dried in the greenhouse, and placed outdoors during rainy weather. After exposure to measured amounts of rainfall the plants were returned to the greenhouse and inoculated.

Four to 6 days after inoculation of detached hop leaves and 8 to 12 days after inoculation of onion plants, infection was determined by the sporulation following overnight incubation in moist chambers. In all tests a high proportion of the check leaves showed sporulation over most of the inoculated leaf area, and the number and epidermal area of the sprayed leaves showing sporulation was proportionately less, according to the efficiency of the fungicide. In the case of the onion, a sprayed leaf or entire plant was treated as a unit and a leaf that showed sporulation over only a fraction of its length was rated as infected, just as would be a leaf infected throughout its entire length. For this reason the figures of percentage infection may indicate much less protection than was actually secured.

#### THE TOXICITY OF SULPHUR AND ROSIN TO ONION MILDEW SPORANGIA

The lack of inhibition of germination, when suspensions of sporangia of *Phytophthora infestans* (Mont.) de By. (6), *Bremia lactucae* E. Regel (5) and *Peronosplasmopara cubensis* (B. and C.) Clint. (2) were placed on sulphur-dusted slides, has indicated the nontoxicity of sulphur to these downy-mildew fungi. It seems, however, that the nature of the substrate or supporting medium has a bearing on this. In the writer's tests, clean glass slides and plates of cold 1 per cent plain agar were dusted simultaneously with sulphur dust (Flottox). Drops of a suspension of onion mildew sporangia were added to the former and as an atomized spray to the latter. These preparations were incubated in the dark at 10° C. in 1 test, at 19° C. in another, and at 22° C. in 2 tests. In these 4 tests on different days, the germination, after 24 hours, was 95, 93, 64, and 86 per cent on check non-treated slides, 75, 78, 69, and 83 per cent on slides dusted with sulphur dust, 91, 92, 51, and 84 per cent on check agar plates, and 0 per cent on sulphur-dusted agar plates.



In another test, small heaps of sulphur dust and drops of a water suspension of it were applied to localized areas on plates of cold agar before the plates were dusted with onion-mildew sporangia. There was no germination for a distance of 1.3 mm. from the edge of the sulphur.

The toxicity of lime-sulphur, copper sulphate, and rosin solutions was tested by adding suspensions of sporangia to solutions of known strength and placing drops of this suspension on glass slides or atomizing these suspensions onto plates of agar. The lowest concentration at which spore germination did not occur was 1-10,000 for lime sulphur on slides and agar plates, 1-100,000 for  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  on slides and agar plates, and 1-10,000 for rosin on slides. On agar plates spores germinated in solutions of 1-1000 rosin.

Thus, sulphur dust, lime sulphur, copper sulphate, and rosin are each shown to be toxic to onion-mildew sporangia, but the toxicity varied greatly with the substrate.

#### INITIAL SPRAY DEPOSIT

Great differences in the amount of fungicide deposited on leaves are apparent to the eye. Since this amount of spray deposit may be an important control factor, the amount of Bordeaux, lime sulphur, rosin, and rosin-lime sulphur deposited on onion, hop, and bean foliage was determined. A weighed and measured leaf was held in a horizontal position, sprayed on both sides with the test fungicide until the maximum deposit seemed to have been reached, drained in a vertical position for about 10 seconds, and weighed. From the weight of wet spray, the known dry matter content of the spray, and the area of the leaf (width of flattened onion leaf multiplied by its length, or planimeter measurement of hop and bean leaves), the amount of fungicide per unit area of leaf was calculated. Thus, in all cases, the sprayed leaf area was twice the measured leaf area, and the spray deposit per unit area of leaf surface would be approximately half that recorded. In determinations of the initial deposit of 14 liquid fungicides by this method the average deviation of a single determination from a mean of 4 determinations was 16 per cent of the mean value.

Visual comparisons of the spread of different sprays on foliage are difficult to make, but a rough index of the relative coverage by the materials used is given in figure 1. "Poor" indicates that the adhering spray formed large or small rounded drops on the leaf surface, with many unwetted areas between the drops. "Excellent" indicates that a smooth uniform film of the fungicide was left on the leaf and there were no unwetted areas. "Fair" indicates a coverage intermediate between these extremes.

Certain very large differences between fungicides are apparent from the data (Fig. 1). On onion leaves, ordinary fungicides do not spread well; but, in spite of this, the writer's experiments showed a heavy total deposit of






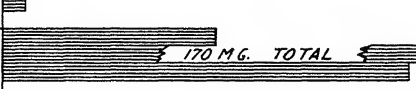
FUNGICIDE	DRY MATTER IN SPRAY %	TEST PLANT	MILLIGRAMS OF DRY MATTER DEPOSITED PER SQUARE DECIMETER OF LEAF												RELATIVE COVERAGE BY SPRAY	
			10	20	30	40	50	60	70	80	90	100	110	120	UPPER LEAF SURFACE	LOWER LEAF SURFACE
1% BORDEAUX	2.3	ONION HOP BEAN													POOR EXCELLENT FAIR	POOR FAIR
1% BORDEAUX + 0.2% PENETROL	2.4	ONION HOP BEAN													EXCELLENT EXCELLENT EXCELLENT	EXCELLENT EXCELLENT
2% LIME SULPHUR	1.0	ONION HOP BEAN													POOR FAIR POOR	POOR POOR
2% LIME SULPHUR 0.5% S.O.S.	1.0	ONION HOP BEAN													EXCELLENT EXCELLENT EXCELLENT	EXCELLENT FAIR
1% ROSIN	1.2	ONION HOP BEAN													EXCELLENT EXCELLENT EXCELLENT	EXCELLENT EXCELLENT
1% ROSIN + 2% LIME SULPHUR	2.2	ONION HOP BEAN													EXCELLENT EXCELLENT EXCELLENT	EXCELLENT EXCELLENT

FIG. 1. Initial deposit and relative coverage by sprays on onion, hop, and bean leaves. Each value is the average of 4 to 12 determinations.

fungicide left in spots over the leaf surface. With Bordeaux mixture, about twice as much fungicide was deposited on onion leaves when no spreader was used as when Penetrol was applied as a spreading agent, but, as will be shown later in inoculation tests, this fungicide gave protection only when employed with a spreader. In the case of lime sulphur, 5 times as much was deposited in the absence of a spreader as when a spreader was used; but here, again, more protection was offered by the smaller amount of fungicide deposited when an adequate spreader was employed. With rosin-lime sulphur the coverage was excellent, and the deposit of spray was about twice that of any other material and 4 times that of any other that spread equally well. On hop and bean leaves, which are much less difficult to wet than onions, the spray deposit was greater than on onions, and spreaders had a less pronounced effect.

#### ROSIN AND LIME SULPHUR AS PROTECTIVE SPRAYS FOR THE CONTROL OF ONION MILDEW

In the writer's tests satisfactory control of onion mildew and increased yield of field onions were secured with lime-sulphur and rosin-lime-sulphur sprays. The results of 2 sets of plots are summarized in table 1. At Berkeley 10 of the check plots and 2 of each of the sprayed plots consisted of 5 evenly spaced plants, each. These were inoculated artificially 6 times between January 6 and April 2. Infection was heavy and fairly uniform in the check plots; in the sprayed plots it was very light up to April 26, and fungicidal control on leaves and young seed stalks was pronounced with all

TABLE 1.—*Field tests of 4 fungicides applied at Berkeley and Alameda, California, for the control of downy mildew of onion*

Fungicide	Berkeley, onions grown for seed, artificial inoculation		
	Replications of treatment	Average of seed stalks infected June 13	Average of uncleaned seed per plant
	No.	Per cent	grams
Control .....	12	84	1.41 ± .22
1% Bordeaux + 0.5% Penetrol .....	4	85	1.30 ± .35 <sup>a</sup>
1% rosin .....	2	76 <sup>b</sup>	.65 ± .25 <sup>b</sup>
2% lime sulphur + 0.05% S.O.S. ....	4	46	6.25 ± 1.69 <sup>a</sup>
	Alameda, onions grown for bunching, natural infection		
	Replications of treatment	Average of leaves infected Nov. 23	Average green weight of plants in 2 ft. of row Dec. 12
Control .....	4	81	170 ± 13
1% Bordeaux + 0.5% Penetrol .....	4	38 <sup>d</sup>	208 ± 16 <sup>d</sup>
2% lime sulphur + 0.05% S.O.S. ....	4	65 <sup>d</sup>	198 ± 15 <sup>d</sup>
Rosin + 2% lime sulphur .....	4	25 <sup>d</sup>	278 ± 21 <sup>d</sup>

<sup>a</sup> Average for 3 spray-application schedules. One replication of plots was sprayed weekly from Jan. 7 to May 4, one fortnightly from Jan. 7 to May 4, and two were sprayed March 11, 31, April 17, and May 4. In the Bordeaux-treated plots those sprayed weekly or fortnightly yielded an average of 0.6 gram of seed per plant, while those sprayed only 4 times produced an average yield of 2.0 grams. Apparently, frequent Bordeaux applications resulted in injury. Plots sprayed weekly or fortnightly with lime sulphur, on the other hand, yielded 9.3 grams per plant, while those sprayed but 4 times yielded but 4.2 grams per plant.

<sup>b</sup> Average for 2 spray-application schedules. One plot was sprayed weekly, the other fortnightly.

<sup>c</sup> In 2 plots 0.5 per cent rosin was used; in 2 others 1.0. Heavier deposit and better control were secured with 1 per cent rosin.

<sup>d</sup> Average for 2 spray-application schedules. Two replications of plots were sprayed Oct. 23 and Nov. 3, and 2 others on Oct. 23, Nov. 3, and Nov. 12. On the average of all treatments the mildew infection was 43 per cent higher and the yield 3 per cent lower in the plots not sprayed on Nov. 12.

materials listed (data not given in table 1). In May most of the sprayed plants became infected and the period of infection continued after the data of last spray application. Differences between plots sprayed weekly and fortnightly were slight and apparently insignificant. Two of the check plots, 2 plots sprayed with Bordeaux, and 2 sprayed with lime sulphur, consisted of 25 plants each. These were inoculated 6 times and sprayed 4 times. Considering all plots together, only the lime-sulphur spray resulted in a significant decrease in infection and increase in yield, and the fungicidal control was apparently greater where lime sulphur was applied weekly or fortnightly than where applied only 4 times.

In the Alameda plots 2 replications of each treatment consisted of 2 4-foot rows and 2 other replications of two 33-foot rows with an average of 32 plants

per linear foot of row. The first spray application was made Oct. 23, when about 25 per cent of the plants showed mildew infection. The second and third spray applications were made Nov. 3 and 12, respectively. No development of the disease was evident between Oct. 23 and Nov. 12, but between Nov. 3 and 23, the disease became epidemic and every nontreated plant observed showed infection. All plots sprayed Oct. 23, Nov. 3, and Nov. 12 showed much less infection than the checks; but where the Nov. 12 application of Bordeaux and lime sulphur was omitted, the plants became heavily infected. All treated plots yielded more than the checks and the yield increase was greatest with rosin-lime sulphur.

The results of greenhouse tests of protective fungicides for onion mildew are summarized in table 2. Bordeaux mixture or lime sulphur alone gave

TABLE 2.—*Greenhouse tests of fungicides applied as sprays for control of downy mildew of onion; plants artificially inoculated the same day they were sprayed*

Fungicide	Independent tests <sup>a</sup>	Tests in which infection occurred	Total leaves inoculated	Average of leaves infected
	No.	No.	No.	Per cent
Control, no fungicide .....	10	10	200	81
1% Bordeaux .....	4	4	79	68
0.2% Bordeaux + 0.2% Penetrol .....	5	2	72	7.6
1.0% Bordeaux + 0.2% Penetrol .....	8	1	132	4.1
2% lime sulphur .....	4	3	82	12
0.4% lime sulphur + 0.05% S.O.S. ....	4	3	72	28
2.0% lime sulphur + 0.05% S.O.S. ....	8	1	141	4.1
Sulphur dust (Flottox) .....	3	3	54	86 <sup>b</sup>
0.2% rosin .....	3	2	37	19
1.0% rosin .....	7	3	121	6.6
0.2% rosin + 0.4% lime sulphur .....	2	2	31	6.5
0.5% rosin + 1.0% lime sulphur .....	2	0	29	0
0.5% rosin + 2.0% lime sulphur .....	2	0	44	0

<sup>a</sup> In every fungicide test here reported the nonsprayed control leaves became abundantly infected.

<sup>b</sup> Though this value indicates higher infection of sulphur-dusted leaves than of control leaves, the infection of the latter was higher in each of these 3 tests.

poor control, but with adequate spreaders and a sufficient concentration of the fungicide, there was complete protection in most of these tests in which the plants were inoculated the same day they were sprayed. Heavy infection, however, frequently resulted when these plants grown from bulbs were inoculated 1 to 5 days after spraying, presumably because of the rapid growth of onion leaves from below, whereby as much as an inch of unsprayed tissue of a single leaf may be exposed in 24 hours.

TABLE 3.—Weathering tests on the efficacy of certain fungicides in prevention of downy mildew of onion showing percentage of plants infected following artificial inoculation. 1936-37

ate test as made	Dec. 14		Dec. 30		Jan. 12						Jan. 28		Feb. 4		Feb. 24			Mar. 9		Average or total
in hours nts were doors	21	21	144	144	53	53	130	130	152	152	122	170	6	30	27	27	51	3	51	73 ave.
int ches) of ural rain which nts were posed	0 <sup>a</sup>	.17	0	0.51	0	0.46	0	0.63	0	0.86	0.94	1.80	1.37	1.92	0	0.99	1.07	0	0.13	0.57 ave.
ber of nts in t	182	168	481	335	115	91	89	113	111	103	254	141	163	165	134	183	123	87	87	3125 total
ntrol (no ngicide)	94	100	100	100	96	100	100	100	95	83	99	100	100	100	100	100	88	100	100	98 ave.
Bordeaux .2% metrol	0	0	0	0	50	0	77	43	72	28	15	12	9	4	.....	.....	.....	24	81	26 ave.
rosin	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	27	79	94	8	47	51 ave.
lime sul- ur + 0.05 S.O.S.	0	38	10	90	2	55	64	46	44	78	.....	.....	28	100	53	100	100	11	100	53 ave.
rosin + % lime phur	0	0	0	0	0	0	0	0	0	0	.....	.....	2	24	0	17	0	0	0	2.3 ave.

<sup>a</sup> Plants exposed to 0 rainfall were in rain-proof cages.

Protection from infection when the plants were inoculated the same day they were sprayed (Table 2) may be of little importance under field conditions, where the fungicide should give protection for several days during variable weather. Rain generally is considered the most important factor in loss of fungicide from the plants and consequent decrease in the protective coating of the leaves. Tests were, therefore, conducted in which the sprayed plants were subjected to known amounts of natural rainfall before inoculation. The results of these tests on greenhouse seedling onions are presented in table 3. Only plants sprayed with rosin-lime sulphur were adequately protected in these tests. Bordeaux deposit was evident even after sprayed plants were exposed to 2 inches of rain; but, as shown by these results, a conspicuous spray deposit did not insure protection.

The results shown in table 3 appear not to accord with those in table 2, in that Bordeaux and lime sulphur generally gave complete protection when bulb plants were sprayed and inoculated the same day, but these same sprays frequently failed to give complete protection on seedling plants, even though the sprays were not subjected to rain. The writer cannot explain this apparent discrepancy, but the greater susceptibility of seedling plants, and the longer time from spraying to inoculation may in part account for the results.

TABLE 4.—*Field tests of Bordeaux, rosin, and lime sulphur for the control of downy mildew of hop. Results expressed in average number of lesions per leaf*

Dates when sprayed, inoculated, and counted	Check	Bordeaux	1 per cent rosin	2 per cent sulphur + 0.05 per cent S.O.S.
1935. <sup>a</sup> Sprayed May 30, inoculated June 1, counted June 5 .....	92 ± 0.1	3.2 ± 0.1 <sup>c</sup>	13 ± 9	
Sprayed June 6, inoculated June 8, counted June 13, .....	97 ± 0.1	13 ± 3 <sup>d</sup>	20 ± 4	
1936. <sup>b</sup> Sprayed May 13, natural infection counted May 27	10 ± 1	0.17 ± 0.04	1.2 ± 0.3	0.15 ± 0.05

<sup>a</sup> Average determined from 8 leaves from each of 4 plots in each treatment.

<sup>b</sup> Determined from counts on 4 leaves from each of 18 plants for check, and 4 leaves from each of 12 plants for each spray plot.

<sup>c</sup> 0.5 per cent Bordeaux + 0.25 per cent rosin fish oil soap.

<sup>d</sup> 1.0 per cent Bordeaux + 0.5 per cent Penetrol.

#### ROSIN AND LIME SULPHUR AS PROTECTIVE SPRAYS FOR THE CONTROL OF HOP MILDEW

In 1935 field hops at Santa Rosa were experimentally sprayed with fungicides on May 16, 23, 30, and June 6. Each fungicide was applied to 4

plots of 10 hills each. Only the May 16 application of 0.5 per cent Bordeaux caused noticeable leaf injury. There were no rains, and almost no natural infection resulted. The plants, therefore, were inoculated artificially and infection resulted as is shown in table 3. Most of the infection occurred on leaves or portions of leaves where no deposit of fungicide could be seen.

Field results were secured from only one application of sprays in 1936. Plants almost free of mildew were sprayed May 13, and a heavy natural inoculation the evening of the same day. Leaves sprayed with Bordeaux or with lime sulphur were noticeably injured. Fungicidal protection was pronounced in all tests (Table 4).

The results of greenhouse tests of protective fungicides for hop mildew are summarized in table 5. None of the sprays caused foliage injury, and

TABLE 5. *Greenhouse tests on the efficacy of certain fungicides for prevention of downy mildew of the hop on detached leaves<sup>a</sup>*

Fungicide	Number of independent tests	Number of tests in which infection occurred	Total number of leaves inoculated	Average relative mildew development per leaf <sup>b</sup>
Control (no fungicide) .....	8	8	22	9.2
0.05% Bordeaux + spreader <sup>c</sup> .....	4	4	9	3.6
0.1 % Bordeaux + spreader <sup>c</sup> .....	2	1	3	0.5
0.2 % Bordeaux + spreader <sup>c</sup> .....	8	3	22	0.22
1.0 % Bordeaux + spreader <sup>c</sup> .....	1	1	3	0
0.05% lime sulphur + 0.05% S.O.S. ....	2	2	6	4
0.2 % lime sulphur + 0.05% S.O.S. ....	6	4	19	0.67
1.0 % lime sulphur + 0.05% S.O.S. ....	2	1	7	0.15
Sulphur dust .....	2	1	5	0.15
0.05% rosin .....	3	3	7	2.3
0.1 % rosin .....	4	2	9	1.5
0.2 % rosin .....	5	3	12	0.26
1.0 % rosin .....	1	1	3	0
0.1 % rosin + 0.2% lime sulphur .....	2	0	6	0

<sup>a</sup> Detached hop leaves from field plants were sprayed on their lower surfaces with the test fungicide, placed with their petioles in vials of water, and inoculated after the fungicide had dried.

<sup>b</sup> 10 indicates heavy infection with 100 or more lesions per leaf; 1 indicates 10 or less lesions per leaf, 0 indicates no observed infection.

<sup>c</sup> 0.25 per cent rosin-fish-oil soap was used as spreader in the 1935 tests; 0.2 per cent Penetrol was used as spreader in the 1936 tests.

protection against the disease was secured with much lower concentrations than was necessary for control of onion mildew. Figure 2 illustrates the effectiveness of dilute rosin-lime sulphur in the control of the downy mildew of the hop.

#### THE EFFECT OF FUNGICIDES ON SPORULATION OF ONION AND HOP MILDEW

Doran (6) found that sulphur dust effectively inhibited sporulation of the cucumber downy mildew fungus, while copper dusts did not. Results of the

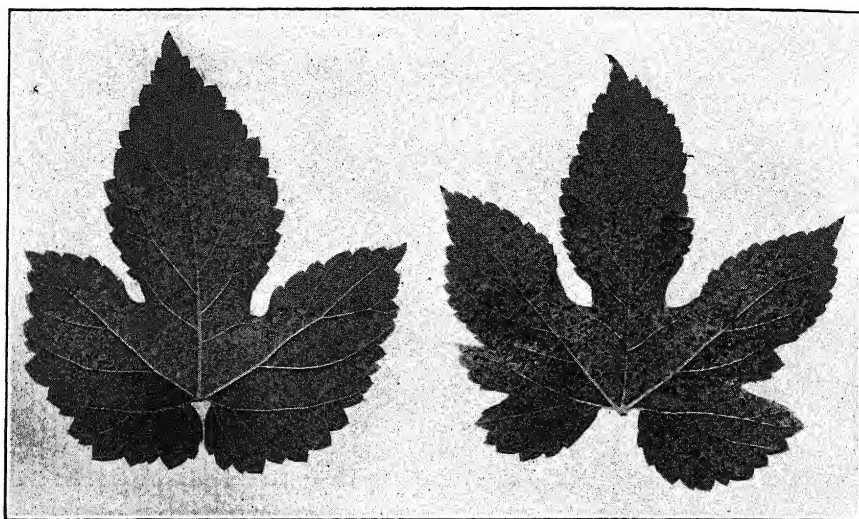


FIG. 2. Protection against downy mildew of hop with dilute fungicides. Hop leaf on left was sprayed with 0.1 per cent rosin + 0.2 per cent lime sulphur at 1:30 p.m. on August 26, 1936. Leaf on right was not sprayed. Both were inoculated 5 hours later. Photograph taken 6 days after inoculation.

writer's tests to measure the comparative effect of fungicides on the sporulation of the causative organisms of both onion and hop mildew are given in table 6. Bordeaux, rosin, and sulphur dust were only slightly inhibitory. Lime sulphur prevented sporulation in most and rosin-lime sulphur in all instances.

TABLE 6. *The effect of certain fungicides on the overnight sporulation of the fungi causing downy mildew of onion and hop*

Fungicide	Onion mildew			Hop mildew, one test with 3 leaves in each test unit. Relative sporulation <sup>a</sup>
	Number of tests	Total number of infected leaves treated	Average relative sporulation <sup>a</sup>	
Control: no fungicide .....	8	15	9.8	10
1% Bordeaux + 0.2% Penetrol .....	2	4	4.5	4
2% lime sulphur + 0.05% S.O.S. ....	4	8	1.2	0
Sulphur dust .....	2	4	9.5	
1% rosin .....	5	10	3.2	8
1% rosin + 2% lime sulphur .....	4	8	0	0

<sup>a</sup> 0 indicates no sporulation; 10 indicates maximum sporulation.

#### SUMMARY

Sulphur dust, lime sulphur, and rosin reduced the germination of onion mildew sporangia, but were less toxic than was copper sulphate. Sulphur



dust was nontoxic or only slightly toxic when drops of spore suspension were added to sulphur-dusted slides, but was very toxic to spores dusted on plates of plain agar that had been dusted with sulphur. Toxicity of rosin, on the other hand, was greatly reduced by agar.

Bordeaux and lime-sulphur sprays without spreaders spread poorly on onion foliage. The addition of Penetrol to Bordeaux and of sodium oleyl sulphate to lime sulphur increased the covering properties of the sprays, decreased the amount of fungicide deposited on the leaves, and increased the protective action of these fungicides.

The amount of fungicide deposited on foliage varied greatly with the fungicide and host used. By far the greatest deposit resulted from the application of rosin-lime sulphur.

Bordeaux, lime sulphur, and rosin effectively reduced downy-mildew infection of onion and hop under field and greenhouse conditions. Onions sprayed with rosin-lime sulphur and exposed to known amounts of rain before inoculation, were not infected in most tests, whereas onions sprayed with Bordeaux, rosin, or lime sulphur were heavily infected under similar conditions. Onions for seed showed greater yield and less infection when sprayed with lime sulphur than when sprayed with Bordeaux or rosin. Onions for greens showed greater yield and less infection when sprayed with rosin-lime sulphur than when sprayed with Bordeaux, lime sulphur, or rosin.

Lime sulphur and rosin-lime sulphur were effective in preventing the sporulation of the onion- and hop-mildew organisms. Other sprays were not.

As judged by the initial deposit on the leaves, protection of onions and hops from mildew infection in the greenhouse, resistance to weathering, protection of onions from mildew infection in the field, and yield of green onions in the field, rosin-lime sulphur was the most effective fungicide tested.

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# RELATIVE EFFICIENCY OF RANDOMIZED-BLOCK AND SPLIT-PLOT DESIGNS OF EXPERIMENTS CONCERNED WITH DAMPING-OFF DATA FOR SUGAR BEETS<sup>1</sup>

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## INTRODUCTION

In the split-plot type of experimental design, first proposed by Yates<sup>3</sup>, one-half of each plot receives a treatment different from the other half, this difference being superimposed on the main treatment. This type of experimental design, for example, is commendable if it is desired to ascertain the effect of 5 fertilizer mixtures (main treatments) on the seedling stand in plots sown with treated and nontreated seed (subtreatments). The subtreatments in a split-plot design would be adjacent to each other for each fertilizer mixture, whereas they might be far apart in the randomized-block arrangement (Fig. 1). Thus, the influence of soil heterogeneity should be

RANDOMIZED-BLOCK ARRANGEMENT

B <sub>A</sub>	E <sub>B</sub>	A <sub>A</sub>	C <sub>A</sub>	D <sub>B</sub>	A <sub>B</sub>	B <sub>B</sub>	C <sub>B</sub>	D <sub>A</sub>	E <sub>A</sub>
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SPLIT-PLOT ARRANGEMENT

C <sub>B</sub>	C <sub>A</sub>	A <sub>A</sub>	A <sub>B</sub>	D <sub>A</sub>	D <sub>B</sub>	B <sub>A</sub>	B <sub>B</sub>	E <sub>B</sub>	E <sub>A</sub>
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FIG. 1. Diagrams of a randomized-block arrangement of plots and of a split-plot type of arrangement. Large capital letters refer to fertilizer mixtures (main treatment); small capitals to treated (A) and nontreated (B) seed.

<sup>1</sup> The data presented in this paper were obtained in cooperative investigations by the Division of Sugar Plants, Bureau of Plant Industry, United States Department of Agriculture, and the Division of Plant Pathology and Botany of the Minnesota Agricultural Experiment Station. Paper No. 1491 of the Journal Series of the Minnesota Experiment Station.

<sup>2</sup> The writer wishes to express his appreciation to Dr. F. R. Immer for suggestions in the statistical analyses and to Dr. E. C. Stakman for suggestions in preparation of the manuscript. Acknowledgment is made of assistance rendered by Mrs. Arlene Burgdorf, Senior Statistician of the Works Progress Administration, for assistance in calculation of data herein reported.

<sup>3</sup> Yates, F. The principles of orthogonality and confounding in replicated experiments. Jour. Agr. Sci. [England] 23: 108-145. 1933. Complex experiments. Jour. Roy. Statis. Soc. Suppl. 2: 181-223. [Discussion, pp. 223-247.] 1935.

reduced to a minimum in the former, and comparisons of the effects of seed treatment should be more accurate.

In determining yields, where plant competition and soil heterogeneity are operative for a considerable time, it is known that the experimental error applied to subtreatments is usually lower in the split-plot design than in completely randomized plots. But it has not been known definitely whether this would be true also in experiments on the control of damping off and similar diseases, where seedling-stand counts are made about 3 weeks after planting and plant competition has not begun.

The writer, therefore, attempted to obtain definite information on the relative efficiency of randomized-block arrangement and split-plot arrangement in field and greenhouse experiments where the emphasis is on seedling stands.

#### METHODS AND MATERIALS

Data were obtained from 2 uniformity trials with sugar beets made in 1936 in the field at St. Paul and Waseca, Minnesota. The land had been uniformly cropped in previous years. The seed was sown in 20-inch rows at about 20 pounds an acre. At St. Paul the plots were 6 rows wide and 25 feet long; at Waseca they were 4 rows wide and 40 feet long. Seedling counts were made about 3 weeks after planting, at St. Paul in 5 4-ft. lengths of row and at Waseca in 5 5-ft. lengths from the middle 2 rows of each plot.

Data, already available<sup>4</sup> from 2 uniformity trials with sugar beets on two types of plant tables in the greenhouse, were used for the present study, also, with the following exception. In this study the seedling counts in the outside rows of pots surrounding each test were discarded, and the analyses are based on the stands in the remaining pots. This was done to eliminate the influence of heat from vertical coils of heating pipes situated along one side and at one end of each plant table.

Since the data considered were obtained from uniformity trials (*i.e.*, the plots and pots were sown to the same variety of sugar beets and at comparable rates), it is possible, by redistribution of assumed treatments, to analyze the results both as randomized-block and split-plot experiments.

The data were analyzed by the analysis-of-variance method<sup>5</sup>.

#### EXPERIMENTAL RESULTS

##### Field Experiments

The analyses of variance of the seedling-stand data were made on the assumption of 5 hypothetical fertilizer mixtures or main treatments, together with treated and nontreated seed or subtreatments.

<sup>4</sup> LeClerg, E. L. Factors affecting experimental error in greenhouse pot tests with sugar beets. *Phytopath.* 25: 1019-1025. 1935.

<sup>5</sup> Fisher, R. A. Statistical methods for research workers. Ed. 4, rev. and enl., 307 pp. Oliver and Boyd, Edinburgh and London. 1932.

In this study it is necessary to compare the magnitude of the variance of subplots within mainplots in the split-plot design with the variance of subplots within blocks in the randomized arrangement. Since the split-plot design places emphasis on the closely contiguous subplots, it is the variance for the subplots within main plots that must be compared with the variance for subplots within blocks of the randomized-block arrangement.

The variance for subplots within main plots in the split-plot design is markedly less than that for subplots within blocks in the randomized arrangement (Table 1). Thus, the split-plot design was 71 per cent more efficient at St. Paul and 53 per cent more at Waseca.

For comparison of main plots within blocks, however, there is a decrease in efficiency by use of the split-plot design (Table 1). This is to be expected,

TABLE 1.—*Analyses of variance of seedling stands in uniformity trials made at St. Paul and Waseca, Minnesota, in 1936*

Variation due to	Degrees of freedom	Mean square or variance at	
		St. Paul	Waseca
<i>Randomized arrangement</i>			
Blocks .....	4	454.55	13,618.28
Subplots within blocks .....	45	821.65	1,590.28
Total .....	49		
<i>Split-plot arrangement</i>			
Blocks .....	4	454.55	13,618.28
Main plots within blocks .....	20	1,249.29	2,283.35
Main plots .....	24		
Subplots within main plots .....	25	479.54	1,035.82
Total .....	49		

as the precision in such a field design is placed on the subplots at the sacrifice of the main plots.

These results indicate that damping-off fungi are not uniformly distributed in the soil and that the split-plot design increases the efficiency of the experiments by reducing variability due to this condition.

#### Greenhouse Experiments

The analyses of variance of the stand counts for each of the pots on the concrete bed were made on the assumption of 19 hypothetical fertilizer mixtures or main treatments, together with treated and nontreated seed or sub-treatments. For the analysis of variance of the stand counts on the board-wall bench, it was necessary to assume 17 hypothetical fertilizer mixtures, together with treated and nontreated seed.

These analyses are given in table 2 and indicate again that compensation

TABLE 2.—*Analyses of variance of seedling stands in uniformity trials made with sugar beets on a raised concrete bed and a board-wall bench in the greenhouse at St. Paul, Minnesota, in 1933*

Variation due to	Degrees of freedom		Mean square or variance	
	Concrete bed	Board-wall bench	Concrete bed	Board-wall bench
<i>Randomized-block arrangement</i>				
Blocks .....	7	9	1,332.00	1,801.50
Subplots within blocks .....	296	330	153.46	211.33
Totals .....	303	339		
<i>Split-plot arrangement</i>				
Blocks .....	7	9	1,332.00	1,801.50
Main plots within blocks .....	144	160	161.18	229.09
Main plots .....	151	169		
Subplots within main plots .....	152	170	146.14	194.61
Totals .....	303	339		

for lack of uniformity is better made by a split-plot arrangement of pots than by a randomized-block arrangement. Thus, the variance for a randomized-block arrangement was 153.46 on the concrete bed and 211.33 on the board-wall bench, whereas, for a split-plot design, the variance was 146.14 and 194.61, respectively, for the 2 types of plant tables. This constitutes an increase in efficiency of 5 per cent on the concrete bed and 9 per cent on the board-wall bench for comparisons of subtreatments. While the magnitude of increase was not so great in the greenhouse tests as in the field tests, yet it is significant that the trend of efficiency was the same in both cases.

#### SUMMARY

From the results herein reported, it is apparent that a split-plot design is more efficient than a randomized-block arrangement for damping-off tests in the field and the greenhouse.

The results also indicate that soil-borne pathogens are not uniformly distributed in the field.

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## SOME BACTERIAL DISEASES OF PLANTS IN ILLINOIS

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Some bacterial-leaf-spot diseases of plants were observed and collected in Illinois in 1929 and the characteristics of the causal organisms from pure culture isolations were investigated in 1929-30 as a graduate research problem<sup>2</sup> by the senior author. Procedures given in the *Manual of Methods for the Pure Culture Study of Bacteria* and the classification in *Bergey's Manual of Determinative Bacteriology, 1930*, were followed in characterizing the organisms. Morphological and biochemical properties of the bacteria that proved to be pathogenic upon artificial inoculation to their respective hosts and general symptoms of the diseases follow.

The symptoms of these diseases in general were brown necrotic leaf spots of circular and angular areas, which were dark and oily and very much shrunken. Early stages of the lesions were small water-soaked areas without any visible necrosis. From free-hand sections mounted in water, oozing of bacterial masses from the sectioned area at the junction of healthy and diseased tissues was observed microscopically in all cases. Stained microtome sections revealed bacteria between the cells of the area of lesions. Typical symptoms developed from the artificial inoculations which were made by atomizing suspensions of the pathogens to the leaves of respective hosts after 24 hours in a moist chamber. Reisolations from these lesions yielded typical cultures.

*Phytopomonas polygoni* n. sp., *P. plantaginis* n. sp., and *P. colurnae* n. sp. are proposed for the pathogens on *Polygonum convolvulus* L., *Plantago lanceolata* L., and *Corylus colurna* L. (Turkish Hazelnut), respectively, since the host relationship and determined characteristics of these organisms are different from other described pathogens. The pathogen on *Cichorium intybus* L., having the same host and being identical with the limited characterization of *Phytopomonas cichori* Swingle, 1925,<sup>3</sup> is considered to be this organism. *Phytopomonas (Bacterium) helianthi* var. *tuberosi* n. var. is proposed for the pathogen on *Helianthus tuberosus* L., since it is quite similar to *Bacterium helianthi* Kawamura, 1934,<sup>4</sup> differing from the latter by failing to peptonize milk, to reduce litmus, and to produce acid from sucrose and glycerol.

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<sup>2</sup> Under Dr. W. F. Tanner, Department of Bacteriology, University of Illinois, Urbana, Illinois.

<sup>3</sup> Swingle, D. B. Center rot of "French endive" or wilt of chicory (*Cichorium intybus* L.). (Abstract) *Phytopath.* 15: 730. 1925.

<sup>4</sup> Kawamura, E. Bacterial leaf spot of sunflower. *Ann. Phytopath. Soc. Japan* 4: 25-28. 1934.

## TECHNICAL DESCRIPTIONS

*Phytomonas polygoni* n. sp. is a short rod 0.5 to 1.5 by 1.5 to 2.5  $\mu$ ; in pairs and single, with rounded ends and no irregular forms; motile by 2 to 8 bipolar flagella; capsulate, but forms no endospores or granules; Gram-positive and not acid-fast; its abundant, filiform, flat, dull, smooth, opaque, pale olive-grey growth on dextrose agar is butyrous with no characteristic odor, but colors the medium brown; colonies are convex with amorphous internal structure and entire margin; in broth it forms strong clouding with pellicle and abundant compact sediments; on gelatin stabs growth is at the top of puncture with stratiform liquefaction and the medium is colored brown; in litmus milk it produces an alkaline reaction and peptonization, but does not reduce litmus or produce coagulation; is aerobic and does not hydrolyse starch, reduce nitrates, or produce indol or hydrogen sulphide; its optimum, minimum, and maximum temperatures and H-ion concentrations for growth are 18, 7, 35° C. and pH 7.5, pH 4.1, and pH 11.0, respectively; its thermal death time is 50° C. for 10 minutes; basic fuchsin, methyl violet, and mercurochrome are toxic at dilutions of  $1 \times 10^{-3}$ ,  $5 \times 10^{-6}$ , and  $1 \times 10^{-5}$ , respectively; it does not produce an appreciable amount of gas from xylose, rhamnose, glucose, mannose, galactose, lactose, fructose, maltose, sucrose, rhaminose, raffinose, dextrin, inulin, glycerol, manitol, sorbitol, ducitol, or salicin; is pathogenic by artificial inoculation to leaves of *Polygonum convolvulus* L.

*Phytomonas plantaginis* n. sp. is a short rod, 0.6 to 1.0 by 1.0 to 1.8  $\mu$ , in pairs, in chains, and single with rounded ends and no irregular forms; motile by 1 and 2 polar flagella; capsulate, but forms no endospores or granules; Gram-negative and not acid-fast; its moderate filiform, raised, opaque yellow growth on dextrose agar is viscid with no coloring of the medium or characteristic odor; colonies are convex with finely granular internal structure and entire margin; in broth it forms moderate clouding with ring and moderate amount of viscid sediment; on gelatin stabs growth is at the top of puncture with slight liquefaction, but no coloring of the medium; in litmus milk it causes no coagulation or reduction of litmus, but produces peptonization and slight acidity; is aerobic and hydrolyses starch, but does not reduce nitrates or produce hydrogen sulphide or indol; its optimum, minimum, and maximum temperatures and H-ion concentrations for growth are 25, 12, and 35° C. and pH 7.2, pH 6.1, and pH 9.0, respectively; its thermal death time is 50° C. for 10 minutes; methyl violet, crystal violet, Dahlia, basic fuchsin, malachite green, brilliant green, phloxine, erythrosin, and acridine yellow are toxic at a dilution of  $1 \times 10^{-3}$ ; it does not produce an appreciable amount of gas from xylose, glucose, lactose, or glycerol; is pathogenic by artificial inoculation to leaves of *Plantago lanceolata* L.

*Phytomonas columnae* n. sp. is a short rod 0.8 to 1.0 by 1.0 to 1.8  $\mu$ , in chains, in pairs, and single with rounded ends and irregular forms; motile by 1 and 2 polar flagella and capsulate, but without endospores or granules; Gram-negative and not acid-fast; on dextrose agar the filiform, raised, dull, smooth, opaque growth is viscid without any characteristic odor or coloring of medium; colonies convex with finely granular internal structure and entire margin; in broth, forms moderate clouding with ring and moderate amount of viscid sediment; on gelatin stabs growth is at the top of puncture with liquefaction, but no coloring of medium; in litmus milk peptonization is complete with production of acid, but no reduction of litmus or coagulation; aerobic; hydrolyses starch, but does not reduce nitrates or produce hydrogen sulphide or indol; its optimum, minimum, and maximum temperatures and H-ion concentrations for growth are 21, 5, and 35° C., and pH 7.2, pH 6.1, and pH 10.0, respectively; its thermal death time is 50° C. for 10 minutes; methyl violet, crystal violet, Dahlia, basic fuchsin, malachite green, brilliant green, phloxine, erythrosin, and acridine yellow are toxic at a dilution of  $1 \times 10^{-3}$ ; it does not produce an appreciable amount of gas from xylose, glucose, sucrose, or glycerol; pathogenic by artificial inoculation to leaves and young stems of *Corylus colurna* L. (Turkish Hazelnut).

*Phytomonas cichorii* Swingle, 1925 is a short rod 0.5 to 0.8 by 1.0 to 1.5  $\mu$ , in chains, in pairs, and single with rounded ends; motile by 1 and 2 polar flagella and capsulate, but forms no endospores, granules, or irregular forms; Gram-negative and not acid-fast; on dextrose agar the moderate, filiform, raised, opaque, dull, smooth, yellow growth is viscid, with no coloring of the medium or characteristic odor; colonies are convex with finely granular internal structure and entire margin; in broth it forms moderate clouding with ring and a moderate amount of viscid sediment; on gelatin stabs growth is at the top of puncture with no liquefaction or coloring of medium; in litmus milk it produces peptonization, but no reduction of litmus or coagulation and the reaction is unchanged; aerobic; does not hydrolyse starch, reduce nitrates, or produce hydrogen sulphide or indol; its optimum, minimum, and maximum temperatures and H-ion concentrations for growth are 25, 12, and 35° C., and pH 7.2, pH 6.1, and pH 9.0, respectively; its thermal death time is 52° C. for 10 minutes; methyl violet, crystal violet, Dahlia, basic fuchsin, malachite green, brilliant green, phloxine, erythrosin, and acridine yellow are toxic at a dilution of  $1 \times 10^{-3}$ ; it does not produce an appreciable amount of gas from xylose, glucose, lactose, sucrose, or glycerol; pathogenic by artificial inoculation to leaves of wild *Cichorium intybus* L.

*Phytomonas helianthi* var. *tuberosi* n. var. is a short rod, 0.5 to 1.0 by 1.5 to 2.5  $\mu$ , in chains and in pairs with rounded ends, but without irregular forms; motile by 2 to 4 polar flagella and capsulate, but without endospores or granules; Gram-negative and not acid-fast; on dextrose agar its glisten-



ing, filiform, flat, smooth, opaque, white growth is butyrous, with no characteristic odor or coloring of the medium; colonies are convex with amorphous internal structure and entire margin; in broth it forms moderate clouding with pellicle and scanty sediment; on gelatin stabs it makes uniform beaded growth along the line of puncture with no liquefaction or coloring of the medium; in litmus milk, causes no coagulation or peptonization or reduction of litmus, but produces an alkaline reaction; facultative anaerobic; does not hydrolyse starch or reduce nitrates or produce hydrogen sulphide or indol; its optimum, minimum, and maximum temperatures and H-ion concentrations for growth are 25, 12, and 35° C., and pH 6.5, pH 4.1, and pH 9.0, respectively; methyl violet, malachite green, and mercurochrome are toxic at a dilution of  $1 \times 10^{-5}$  and basic fuchsin, methylene blue, and eosin at  $1 \times 10^{-3}$ ; it does not produce an appreciable amount of gas from xylose, rhamnose, glucose, mannose, galactose, maltose, sucrose, rhaminose, raffinose, dextrin, inulin, glycerol, manitol, sorbitol, ducitol, or salicin; pathogenic by artificial inoculation to leaves of *Helianthus tuberosus* L.

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## PHYTOPATHOLOGICAL NOTES

*Isolating Single Spores Without Special Equipment.*—Methods for isolating single fungus spores are not rare, but usually require expensive or special equipment. An inexpensive method is here reported that has been used successfully with spores conveniently observable with the low-power objective of the microscope. The procedure is not entirely new, but combines parts of several methods into a simplified form.

The only special instrument needed is a glass transferring rod made as follows:

A small glass rod or tube of small bore is heated in a flame and drawn out to hairlike dimensions. A small knob is produced at the extremity of this glass hair by touching it lightly to the base of the flame. Bending the hair near the tip or further back (Fig. 1, A) facilitates a downward movement, after the instrument has been inserted under a partially raised Petri dish lid. For ease of manipulation, the instrument should have a total length of 3 to 4 inches.

*Method.* A clear, hard agar is poured into a Petri dish and allowed to solidify. Five to 10 circles of approximately 4–5 mm. diameter are then marked on the bottom face of the dish with a wax pencil (Fig. 1, B). A thin spore suspension is next made in a drop of sterile water placed on a flamed microscope slide. The tip of the transferring rod is dipped into the spore suspension and lightly touched to the agar surface within the area enclosed by one of the circles. It is desirable to dent the agar surface but not to break it, since spores may then be deposited in several planes. After droplets have been deposited on the agar above all the circles, the inverted dish is placed on the microscope stage and the inoculated areas examined under the low-power objective.

All spores are usually visible in the droplet if examined soon after they are transferred, but they may be slightly beyond the borders of the droplet if allowed to stand for several hours before examination. A single spore usually can be obtained in one or more droplets in each dish, after some practice, unless the worker is handicapped by nervousness or a lack of touch.

Several suggestions may be helpful to those employing this method for the first time:

1. A spore suspension containing 25–200 spores per drop has given best results. This dilution can be obtained with ascomycetes by allowing a single ascocarp in a Van Tiegham cell to discharge its spores into a sterile drop of water on a flamed cover slip. Fewer spores can be obtained in cases of heavy discharge either by shortening the period of discharge or increasing the distance between ascocarp and water drop.

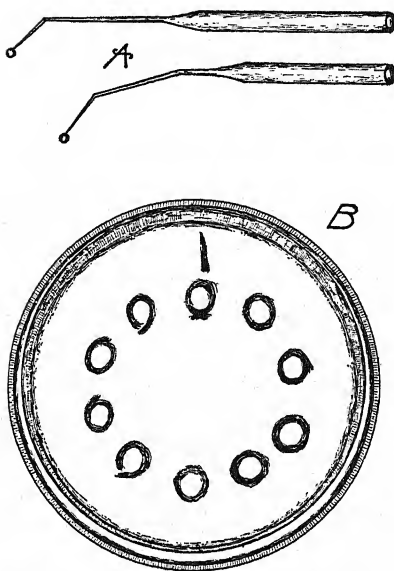


FIG. 1. Materials necessary to isolation of single spores of fungi. A. Glass transferring rods. B. Petri dish containing hard agar on which 10 circles have been made with a wax pencil.

2. If too many or too few spores are consistently being transferred, decrease or increase the size of the knob on the glass hair. Touching the agar over 2 successive circles before redipping in the spore suspension sometimes is effective when too many spores are being transferred.

3. The agar surface generally can be kept unbroken by using a somewhat flexible glass hair; only the knob need be of variable size.

Germination and growth can be easily observed and followed in the drop-lets until colonies are ready to be transferred. Any stray contaminations outside the inoculated areas should then be visible to the naked eye. The colonies may be transferred to tubes or other containers by the method outlined by Keitt,<sup>1</sup> or directly by the usual needle method.

Single-spore cultures of *Gloeosporium perennans*, *Neofabraea malicorticis*, *Venturia pyrina*, *Cucurbitaria berberidis*, and species of *Ciboria* and *Sclerotinia* have been obtained by this method. Some Petri dishes have yielded as high as 90 per cent of single-spore cultures.—J. R. KIENHOLZ, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

*Some Fungous Diseases of Clarkia elegans.*—*Clarkia elegans* Douglas, a native annual plant of California, is highly susceptible to fungous infection during the seedling stage and directly after transplanting. A study was carried on to determine some of the pathogenic forms.

<sup>1</sup> Keitt, G. W. Simple technique for isolating single-spore strains of certain types of fungi. *Phytopath.* 5: 266-269, 1915.

A survey of the literature reveals only 2 publications of fungi on this host; *Vermicularia clarkia* Fautrey on the leaves, and *Cytosporella clarkia* Oudemans on the stems, have been reported for Europe. No reference to their occurrence in America was found. An examination of specimens in the Dudley Herbarium, Stanford University, provides 2 additional fungi, which, to the writer's knowledge, have not been previously reported. *Puccinia clarkia* Peck was collected by E. Braunton at Glendale, California; *Synchytrium fulgens* Schroeter was collected at Lower Goat Ranch Gulch, San Mateo County, California, by James McMurphy.

*Method.* Fungi were isolated from infected plants and grown in pure culture. Inoculation of plants free from infection was necessary to determine those pathogenic to *Clarkia elegans*. Of several methods tried, the hot-water treatment at 50° C. for 30 minutes<sup>1</sup> gave the highest percentage of germination of noninfected plants.

The treated seeds were sown on Knop's agar medium. As fungus growth could readily be seen on the seeds and at the base of the seedlings, it was possible to discard infected seedlings resulting from inadequately treated seeds. A  $\frac{1}{2}$ -inch layer of medium was sterilized in  $2\frac{1}{2} \times 4$  inch jars with lids. After cooling, the jars were inverted, the seeds were planted on the agar, and the lids screwed on. The jars were then placed in a favorable light and maintained at room temperature.

Normal disease-free seedlings averaging  $1\frac{1}{2}$  to  $2\frac{1}{2}$  inches in height, were inoculated with pure cultures of the isolated fungi and the effect noted. Of the 19 fungi found on diseased *Clarkia elegans* plants, all but 5 were seen to affect the seedlings.

*List of Fungi on Clarkia elegans not Previously Reported.* *Alternaria tenuis* Nees, stem and leaves; <sup>2</sup>*Aspergillus niger* Van Tieg, stem; <sup>2</sup>*Aspergillus* sp., stem; <sup>2</sup>*Aspergillus wentii* Wehmer, stem; *Botrytis cinerea* Pers., stem and leaves; *Citromyces griseus* Sopp., leaves; *Cladosporium elegans* Penz., leaves; *Fusarium* sp., stem; *Helminthosporium* sp., leaves; *Hormodendron cladosporioides* (Fres.) Sacc., leaves; <sup>2</sup>*Mucor tenuis* Lind., stem; <sup>2</sup>*Mucor* sp., leaves; *Oospora epilobii* (Cda.) Sacc. et Vogl., stem and leaves; *Penicillium brevicaulis* Sacc., stem and leaves; *Peronospora arthuri* Farlow, leaves; *Pleospora herbarum* (Pers.) Rabh., stem and leaves; <sup>3</sup>*Puccinia clarkiae* Pk., leaves; *Pythium debaryanum* Hesse, stem; <sup>3</sup>*Synchytrium fulgens* Schroeter, stem and leaves; *Verticillium albo-atrum* Reinke et Berth., stem and leaves.

*Summary.* The results of this study show that *Clarkia elegans* seedlings and mature plants are very susceptible to fungous infection. While the controlled conditions of seedling inoculations were not normal for the plant, the symptoms were consistent with reports of similar forms on other and re-

<sup>1</sup> Walker, J. C. The hot water treatment of cabbage seed. *Phytopath.* 13: 251-253. 1923.

<sup>2</sup> Not observed to be pathogenic on *Clarkia elegans*.

<sup>3</sup> Not found in experiment garden, but in the Dudley Herbarium.

lated host plants. The use of greater numbers of seedlings for inoculation and of more mature plants on different substrates and under varied environmental conditions is desirable in order to determine the degree of parasitism of each fungus found on *Clarkia elegans*.—ESTHER A. LEWIS, Stanford University, California.

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## REPORT OF THE TWENTY-FIRST ANNUAL MEETING OF THE PACIFIC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

### THE 1937, DENVER, COLORADO SUMMER MEETING

A joint meeting of the Pacific Division of The American Phytopathological Society and of the Upper Mississippi Valley Group of Plant Pathologists was held in Colorado June 23 to 26, 1937. On instructions from the Council, the Pacific Division assumed charge of the meeting for the presentation of papers held in connection with the summer meeting of the American Association for the Advancement of Science, in Denver. Approximately 40 pathologists and visitors attended the sessions. The Mississippi Valley, the Rocky Mountain area, and the Pacific Coast were about equally represented with 2 members from Washington, D. C. Fifteen papers were presented, in the 3 half-day sessions, and were informally discussed. Interest was added by the discussions of status of the national society and of Phytopathology by G. W. Keitt and H. B. Humphrey.

At the joint session of the Botanical Sciences, held Wednesday afternoon, the pathologists were represented by J. T. Barrett of the University of California, Berkeley, who presented a paper entitled "Studies on Some of the Lower Forms of Parasitic Phycomycetes."

A short business meeting was held Wednesday morning; C. W. Bennett of the U. S. Department of Agriculture, Riverside, California, was elected President and B. F. Dana of the U. S. Department of Agriculture, Corvallis, Oregon, was elected Vice-president of the Pacific Division of The American Phytopathological Society. Other officers will continue to serve until 1938, when a general election will be held at San Diego, California, the next meeting place of the Society.

On Friday, plant pathologists and their families were guests of the Upper Mississippi Valley Group on a tour that included a visit to the experiment station of the U. S. Department of Agriculture at Greeley and to the campus of Colorado State College, Fort Collins, where problems in the construction of dams were demonstrated by means of models in the irrigation hydraulic laboratories.

A considerable number of visitors completed the tour through Rocky Mountain National Park with stops at Estes Park and Grand Lake. At the

former location Dr. C. A. Lory, President of Colorado State College, presented an interesting report on Water Resources and Irrigation Problems in Colorado. Moving pictures of plants and animals in the park also were shown.

L. D. LEACH, *Secretary-Treasurer*.

*Control of Peach Mosaic in Colorado.* E. W. BODINE.

Peach mosaic is being controlled in Colorado by eradication of diseased trees. This disease was found in 1931 in the Palisade region and rapidly increased, the number of diseased trees being approximately squared each year before eradication started. At the peak of eradication over 30,000 trees were taken out in one season.

By careful survey and persistent eradication carried on by the State Experiment Station, the State Entomologist, and the U. S. Bureau of Entomology and Plant Quarantine, cooperating, the number of new cases of diseased trees has been cut to 3,100 this year, giving indication that in the near future the infection may be reduced to a relatively negligible quantity.

*The Maynard Plum—A Carrier of the Peach Mosaic.* E. W. BODINE.

Since 1935 plum trees have been suspected of being carriers of peach mosaic though the plums showed no symptoms of the disease. During the seasons of 1936 and 1937 pieces of root from suspected plums were grafted on healthy peach and buds from plums were budded on healthy peach. These unions were successful and in as short a time as two months the peach trees grafted or budded with Maynard plum showed typical mosaic symptoms. Though all of the plums used did not produce mosaic on the peach, it appears from these tests that they may be carriers of the virus.

*Peach-Mosaic Host-Relationship Studies in Southern California.* L. C. COCHRAN and LEE M. HUTCHINS.

Naturally occurring mosaics have been observed on apricot, almond, prune, plum, and Myrobalan plum in the same districts in Southern California, where peach mosaic occurs. The almond mosaic is extensive in the Banning region and the apricot mosaic is widespread in both the Beaumont and Banning areas. Random infection of the apricot mosaic occurs in the Hemet area. Mosaic on prune and plum has been observed, with a few exceptions only, where the prune or plum was growing on mosaic-affected understock of peach, almond, or Myrobalan plum.

Scions from mosaic-affected trees of apricot, almond, prune, plum, and Myrobalan plum, when placed on healthy J. H. Hale peach nursery trees, produce in leaves symptoms that are indistinguishable from those of peach mosaic induced in other healthy J. H. Hale nursery trees by graft inoculations from mosaic-affected peach trees. Peach scions from trees affected by the peach mosaic disease were grafted in nursery trees of apricot, almond, prune, and plum, but have not yet induced in the inoculated trees symptoms identical with the mosaics occurring in these species in the field.

Mosaic-like symptoms have been induced in a number of *Prunus* species, other than those last mentioned, by inoculation with buds from mosaic-affected peach trees. Reinoculation back to peach is being attempted.

These data establish the fact that certain mosaics of widespread natural occurrence on apricot and almond, and occasionally on prune, plum, and Myrobalan plum are transmissible to peach by grafting and produce symptoms in peach leaves not unlike peach mosaic. Identity of these viruses with peach mosaic, however, should not be regarded with more than strong suspicion until cross-inoculations have been completed.

*History of Elsinoë fawcetti, the Causal Fungus of Citrus Scab.* ANNA E. JENKINS and A. A. BITANCOURT.

*Sphaceloma fawcetti*, although in general appearance atypical for *Sphaceloma*, as originally described, was classified in this genus chiefly on the basis of cultural similarities with the generic type. The recent discovery of the ascomycetous stage provided for its classification in the genus *Elsinoë* as *E. fawcetti*. A part of the mycological history of the fungus may now be prepared and the synonymy given. In 1886, Scribner first reported the organism from Florida, where it is believed to have been of Japanese origin. He designated it *Cladosporium* sp., following Ellis, who examined specimens and wrote

that it was none of Penzig's *Cladosporii* on citrus. Early illustrations are by Scribner, 1886, Swingle, 1893, and Hume, as drawn by McCullough, 1900. In 1906 Fawcett isolated and cultured it, and an exsiccatum was published in *Fungi Columbiana* (*Fungi Columbiana* 2316). He later proved by inoculations that this fungus causes citrus scab. In 1925 Jenkins found the organism to be a *Sphaceloma*, and, continuing the study on this basis, showed conclusively that the species produces on scab lesions a *Cladosporium* type of growth, the *Cladosporium* of Ellis. The previously published description of the perfect stage is based upon the ascomycete, as found by Bitancourt and Jenkins on Satsuma orange fruit from Brazil.

*A Vascular Rhizoctonosis of Sugar Beet.* W. A. KREUTZER.

A sugar-beet root showing a marked vascular necrosis was found in the Rocky Ford district by W. J. Henderson. Isolations from the infected bundles yielded a species of *Rhizoctonia*. Soil-inoculation tests showed that the organism is capable of causing a severe damping off of sugar-beet seedlings and a vascular necrosis of young beet roots. The fungus is weakly parasitic on more mature beet roots.

Histological studies of infected tissue revealed the presence of *Rhizoctonia* hyphae only in the vessels. Formation of a gum-like material in the invaded xylem elements and necrotic changes in the adjacent xylem parenchyma appear to be constant features of the invaded tissue.

*A Phytophthora Rot of Cucumber Fruit.* W. A. KREUTZER.

In the fall of 1936, specimens of rotting cucumber fruits were collected in the Rocky Ford district by W. J. Henderson. The disease was confined to an 8-acre field where 100 per cent of the fruits were infected. Isolations from this material consistently yielded a species of *Phytophthora*, which, on being reinoculated into healthy fruits, produced the rot. The fungus was found to be pathogenic to Hubbard squash fruit and red and green bell pepper fruits, and also was found capable of inducing a severe damping off of cucumber and pepper seedlings.

Because of its similarity in culture to *Phytophthora capsici*, the organism was introduced into soil in which mature peppers were growing. After a period of from 10 to 20 days the plants showed a pronounced blight. The cucumber *Phytophthora* to date has not produced any sexual fruiting bodies, although it has been grown on Tucker's differential media for 6 months. It is probable that the cucumber organism is either a closely related species or a new strain of *Phytophthora capsici*, the causal agent of pepper blight.

*Determination of Sclerotial Populations by Soil Analysis, and Prediction of Losses in Sugar Beets from Sclerotium rolfsii.* L. D. LEACH.

*A Tomato Resistant to Two Wilts.* MICHAEL SHAPOVALOV and J. M. LESLEY.

Certain soils of the coastal belt on the Pacific Coast frequently are infested with *Verticillium albo-atrum* R. and B., as well as *Fusarium lycopersici* Sacc. To meet this situation the United States Department of Agriculture and the University of California have developed cooperatively a new variety of tomato named *Riverside*, which is resistant to both kinds of wilt. It was originated from a cross between Cal 2, somewhat resistant to *Verticillium* wilt, and Marvana, resistant to *Fusarium* wilt. The new hybrid was tested repeatedly on soils infested with both these parasites, and invariably showed much higher resistance to both wilts than any of the several leading commercial varieties tested simultaneously. The fruit of the *Riverside* is red, round, and very firm, is comparable in size to Norton, but, unlike the latter, almost entirely free from cracks under conditions thus far tested. It is suitable for both shipping and canning, but may be regarded primarily as a late shipper because of its late maturity.

The fungus cultures made in the course of these wilt experiments showed that, as a rule, *Fusarium* was more frequent during the hotter part of the growing season, whereas *Verticillium* tended to gain the ascendancy with the advance of cooler weather, not infrequently superseding *Fusarium* in the same plant.

*Black Ring, A Virosis of Cabbage and Other Crucifers.* C. M. TOMPKINS, M. W. GARDNER, and H. REX THOMAS.

Black ring, a virosis of cabbage, occurs during the summer, fall, and winter months in the San Francisco Bay region and in certain coastal and interior valleys of California. Cool weather favors the disease. Early symptoms consist of numerous, chlorotic rings that collectively induce marked leaf chlorosis. The rings later become black and necrotic, and the central tissue in the ring also may die, producing black spots. In the field only

the older cabbage leaves show symptoms. The spots are most conspicuous on the dorsal surface of the leaf. In the greenhouse, systemic infection of healthy cabbage seedlings was obtained, after 9 to 21 days, by rubbing the leaves with expressed juice and carborundum. The insect vectors, which breed naturally on cabbage, are the cabbage and green peach aphids. The virus is inactivated by heating for 10 minutes at 59° C., by aging for 3 days at 22° C., and by diluting 1 to 1000. All commercial varieties of cabbage appear to be susceptible. Infection also was obtained on rhubarb, *Chenopodium album*, *C. murale*, spinach, *Stellaria media*, *Brassica arvensis*, kale, brussels sprouts, cauliflower, broccoli, kohlrabi, rutabaga, turnip, wallflower, annual and Brompton stock, dames violet, Virginian stock, water cress, honesty, Chinese radish. Turkish and White Burley tobacco, and *Nicotiana glutinosa*. This disease is similar to a ringspot disease of cabbage described in 1935 by Kenneth M. Smith in England.

*The Infestation of Soil with Ophiobolus graminis and its Subsequent Increase and Spread in the Soil.* HURLEY FELLOWS.

A study was made to determine by what means *Ophiobolus graminis* is distributed in the soil. Experimental data indicate that any conveyor of soil particles or plant debris may carry infestation. Establishment of take all in a new location is a slow, hazardous process. Greenhouse soil must have at least 25 per cent, by volume, of infested soil to produce appreciable disease on the succeeding wheat crop. Successive crops grown in the same mixture become increasingly more severely diseased. Infestation in a 15 per cent mixture did not increase with 4 years' cropping. Either mixture had a greater concentration of infestation than ordinarily occurs in the field.

Noninfested soil, if contaminated with a water suspension from infested soil, will grow infested wheat plants in the greenhouse if cropped with wheat two years. Infection did not appear during three years' cropping in the field; but when the soil was removed to the greenhouse and cropped, two more years' infection appeared. Noninfested soil, inoculated by applying infested soil lightly to the surface to simulate wind blowing, did not grow diseased plants in the field during three years' cropping; when brought to the greenhouse and cropped two more years, the disease appeared from this source.

*Ophiobolus graminis* did not spread from infested to noninfested soil when such soils were placed in contact without mixing, provided no wheat roots grew through the adjacent layers. When roots did grow through them the fungus spread to noninfested soil, more abundantly when the roots grew from the noninfested into the infested soil than *vice versa*. Infested soils in contact with noninfested often lose their infestation both in field and greenhouse. Infestation may often apparently disappear from take-all spots in the field. Dead, diseased host remains carried infestation in the greenhouse but not in the field. Spores from perithecia of *Ophiobolus graminis*, though a source of infection, are seldom found in the Middle West.

Cultures of *Ophiobolus graminis* grown on a sterilized barley-oat medium provided effective inoculum for field and greenhouse. Soils thus infested long remained so. Infected roots of living host plant are perhaps the most certain carriers of *Ophiobolus graminis* to noninfested soil. This explains why rotation of crops is an effective control for take all.

*Effect of Climatic Conditions on the Prevalence of Ophiobolus graminis in the soil.* HURLEY FELLOWS.

*Ophiobolus graminis* the parasite causing take all of wheat is not killed, when in pure culture, by Kansas winter temperatures, nor is it affected by abrupt changes from growing to subfreezing temperatures. The thermal death point of both micro- and macro-hyphae is 50° C. The high summer temperatures and drought only slightly reduce soil infestation. The abundance of the organism in the soil is not reduced by alterations of the soil from growing temperatures to subfreezing. The abundance of the parasite in infested soil is altered by various combinations of moisture, temperature, and compactness of the soil. In general, cool soils tend to retain the organism and warm ones to lose it. A warm, loose soil retains a minimum of the organism and a cool, compact, moist soil the most.

*Further Studies on Carbohydrates and Nitrate Nitrogen in Psyllid yellows Disease of Irish Potato.* J. R. EYER.

*Recent Studies on the Control of Phymatotrichum Root Rot.* R. B. STREETS.

*Varietal Resistance of Tomatoes to Fusarium Wilt.* P. A. YOUNG.



## THE COPPER CONTENT OF RESIDUES FROM SPRAYS CONTAINING ADJUVANTS

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### INTRODUCTION

An active search is under way for methods, devices, and materials that will assure the deposition of continuous and effective coatings of residues from plant sprays applied as fungicidal protectants. Materials added to the spray liquids may be for the purpose of reducing the surface tension, increasing the wetting power, removing the air film on plant organs, reducing or promoting the activity of surfaces, conferring adhesiveness in the usual sense of the word, or making the film of residue more elastic. Each of these different effects has been observed, although usually not singly.

The effect most often obtained is reduction of the surface tension of the liquid, accompanied frequently by an increase in the wetting power. Without the adjuvant, the spray is deposited in drops and does not form a continuous film unless a deliberate effort is made and considerable time spent in applying the spray directly to even the minutest areas of exposed plant surface. During drying, even though the film may have been continuous, it again breaks up into drops and leaves a splotchy residue. With the adjuvant, the spray forms a continuous film that readily coats all exposed parts and leaves an apparently uniform residue, even when it dries.

Unfortunately, disease control is not correspondingly increased. There have been disappointingly few instances where the addition of the adjuvant has wrought a definite improvement in the control. In a considerable number of cases the nonmodified spray has given as good or even better control of the disease than the one containing the adjuvant.

Many of the substances used as adjuvants are detergents in other fields. This implies that not only do they go to the water-air interface, as shown by the reduction of surface tension, but, also, to the solid-particle-water interface, thus eventually loosening solid foreign particles from their attachments to solids and facilitating their removal by washing processes. If such an adjuvant is added in too liberal quantity, the resulting deposit of active material will be undesirably slight. If the behavior of such a spray liquid is watched closely during application it will be noticed that, although the plant organs are covered readily, all the liquid except an excessively thin film runs off. At first glance it seems that the surface tension of the liquid has been too greatly reduced, but there are also indications that the particles

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are failing to show their usual tendency to cling to the parts of the sprayed plant. This may well be ascribed to the detergent effect. The amount of an adjuvant that should be added to each different spray liquid is, therefore, a matter of some importance. A given adjuvant may fail to produce the desired effect when added in one proportion, but may do much better if tried at another proportion. Studies in the flotation of ores warrant the statement that a substance may show greater affinity for the surface of one solid than another, and thus show greater detergent action for one sort of particle than another. A prime detergent under certain conditions may, therefore, fail to show this effect when used as a spray adjuvant, especially if an excess is avoided, and this can be done only by trial. It is, nevertheless, a distinct anomaly that the same substance should behave with one kind of particle as a detergent and with another as an adhesive; yet, just this claim has been made for some of these materials.

The suggestion that an adjuvant may migrate from free dispersion in the water to the surfaces of the spray particles carries the implication also that the chemical behavior of these particles of active material may be changed by the presence of this coating. Insecticides that are ingested encounter inside the gut of an insect an environment of much greater chemical activity than that prevailing on the surface of the sprayed plant. Like the coating of a pill, such a surface contamination can be readily removed by digestion. Consequently, the great amount of work done with adjuvants for insecticides has not encountered difficulty from this source. Since a fungicide must, however, operate in the environment of the leaf or fruit and be absorbed by the fungus spore from this environment, or not at all, it is evident that an adjuvant that coats the fungicidal particles may decrease the effectiveness of these particles by interfering with absorption. Such an interference by a coating of adjuvant, impervious to the agencies to which it was exposed, has been observed by Branas and Dulac (2), by Farrar (6) and by the writers (unpublished data).

These considerations are of great importance in the development of new fungicides. After a compound is prepared for test as a fungicide, care should be bestowed on its physical properties, such as fineness of grinding, surface activity, and adhesiveness in general, since these properties determine how well a residue of this substance will cover the sprayed plant and how long it will stay in place for the test. Until these properties have been brought to satisfactory conditions, no adequate test of the fungicide is possible. One of us (the junior writer) has developed a promising new material—copper phosphate (9)—as an orchard fungicide, and its uses and limitations are being studied. Naturally, the use of adjuvants has been investigated, and one of these—bentonite flocculated by lime (10)—has been used generally with copper phosphate. This adjuvant does not alter the surface

tension or wetting power of the spray liquid; its presence in the residue is believed to confer a better texture and tenacity. The present work reports tests of the value of several adjuvants for use with the copper phosphate-lime-bentonite mixture, each chosen as typical of a certain class.

A numerical measure of the density of the resulting residue was sought, so that unsupported opinion based on the appearance would not need to be used. It was hoped that certain of these classes could be selected as offering promise for future work.

#### MATERIALS AND METHODS

In 1935, 4 and in 1936, 5 spray mixtures based on copper phosphate were applied to experimental blocks of Kieffer pear trees at the United States Horticultural Field Station at Beltsville, Maryland. All contained 2 pounds of copper phosphate, 4 pounds of hydrated lime, 2 pounds of bentonite, and water to make 50 gallons. One treatment contained no other ingredients; the second contained 4 ounces of a synthetic organic detergent per 100 gallons; the third,  $\frac{2}{3}$  lb. of a new commercially prepared adjuvant; the fourth, 1 pound of a special fish-oil soap, and the fifth, applied only in 1936, contained 1 quart of cottonseed oil per 100 gallons.

The makers of the synthetic detergent described it as a "butylated diphenyl sulfonic acid." It is ostensibly a single, technically pure substance and comes either as a straw-yellow powder of indefinite structure or as a dark brown liquid, a 25 per cent solution of the solid in water. In common with most of the sulfonic acids, it is highly soluble in water and takes water slowly from the air, and, like the sulfonated fatty acids, it shows extreme surface activity in water. It is said to be widely used as a detergent and wetting agent, and a similar product made by the same company has been found useful in the washing of apples to remove arsenical spray residues. Like the sulfonated fatty acids, and in contrast to soap, it is stable chemically and exerts its usual effects in the presence of mineral acid. In this paper it will be called "the aromatic sulfonate."

The commercially prepared adjuvant is a solution containing more than 2 substances. Exposed to air at room temperature, about 20 per cent evaporated, apparently an inert volatile solvent. The viscous liquid remaining lost no more weight. The preparation disperses readily in water, but with a fine turbidity that may be due to the emulsification of the volatile solvent. It is a good emulsifier, does not display such pronounced surface activity as the aromatic sulfonate, and its effects on the behavior of the spray liquid were, in general, less striking than with many proposed adjuvants. A publication from the maker says it is "... A resinous material ... developed synthetically ... combined with sodium oleyl sulfate with suitable blending agents". It will here be referred to as the resin mixture.

The fish-oil soap is said to be made from the fatty acids obtained by a catalytic deesterification of the glycerides of fish oil, a step in the process of isolating vitamin concentrates. It is a dark brown, viscous liquid containing 30 per cent dry soap and has the usual characteristics of such a soap, except that it resists to an unusual degree decomposition by lime. No explanation of this is available.

The cottonseed oil was not a regular commercial product, but was sent by a cotton-oil company upon our request for an average unrefined cottonseed oil, representing such a grade as would be supplied for this purpose, if its use should become established.

The other spray materials were of the grade usually employed for such purposes. The copper phosphate was supplied by a chemical manufacturing company and was a technical product intended for spraying. The lime was a good grade of hydrated lime, made especially for building purposes. The bentonite was a commercial, select, finely ground, natural bentonite. The water was pumped from a small creek tributary to the Anacostia River that runs through the farm of the United States Horticultural Field Station at Beltsville.

The fungicidal-spray program was carried out in this orchard to control the organism (*Fabraea maculata* (Lév.) Atk.) causing pear leaf blight or Entomosporium leaf spot. It had almost defoliated the orchard and destroyed the crop in 1933, and has continued to be active, as shown by the unsprayed plots. It affects both the leaves and the fruits, but, since it is the spores from the leaves that infect the fruit, recording the percentage of infected fruits instead of estimating the percentage of infected leaves was used as a method of determining the degree of control.

Since the effectiveness of none of the combinations based on copper phosphate was known, a standard fungicidal treatment whose effectiveness in the control of this disease has been established was applied to another plot for comparison. One application of 1-40 liquid lime sulphur was made at the time half the petals had fallen, and another about 3 weeks later. The third application, about 3 weeks after the second, was Bordeaux mixture, consisting of 1 pound of crystalline copper sulphate and 3 pounds of lime to 50 gallons of water. In 1935 this application was made in the last week in June. The final application was of the same composition and was put on at the end of July.

Lead arsenate was added at the rate of 1 pound to 50 gallons in all the sprays applied in May of each year.

Next to a determination of the effect of the spray on the disease, the quantity of copper remaining on the sprayed leaves at intervals after application seems to offer a useful criterion of the value of an adjuvant. In addition, the differences in quantities of copper may be of value in explain-

ing the success or failure of adjuvants. Accordingly, sampling was begun in May and continued to October. A number of samples were wasted in 1935 in establishing a procedure to be followed, and desirable modifications were adopted from time to time, especially between the two seasons. Special effort was made to avoid the use of elaborate apparatus and costly operations, so that the most highly recommended procedures could not always be adopted (4, 14). Of the 700 separate determinations in 1935, nearly 500 were shown to be by methods of small dependability, and will not be reported.

To simplify the calculation of the area of leaves, samples of fixed area (8) were obtained by cutting a circle or disk out of a fixed number of freshly picked leaves. At first a 1-in. (25 mm.) brass cork borer was used against a platen of paper towel. Control tests showed that copper from the borer was too little to effect the results, but the constant threat of contamination and the short life of the cutting edge led to the adoption of a steel cutter operating against a piece of thick rubber from the inner tube of a tire. Small shreds of this rubber could not be prevented from occasionally appearing in the samples; but, since the rubber was present in minute quantities and contained only 30 parts of copper per million, this was considered of no consequence.

As the circles were severed from the rest of the leaf they remained in the body of the tube, and, when the required number had been cut, they were removed with steel forceps and placed in a salve box made of lacquered tin plate. These boxes were closed in the orchard and were opened only to transfer the samples to porcelain dishes for incineration. The muffle was heated from room temperature to something below visible redness for 3 hours, always with the same set of the rheostat. The ash obtained in this way gave a colorless solution in dilute nitric acid, although enough copper was present in many of the samples to show the black color of cupric oxide in the ash. The ash was wetted with 5 ml. of water, treated with 10 drops (0.5 ml.) of nitric acid, and evaporated to dryness on the hot plate. The residue was then moistened with enough hydrochloric acid to wet it thoroughly, evaporated to dryness, and baked to insolubilize the silica. It appears reasonable to suppose that the silica will remain inert through subsequent operations in acid solution, and thus can be filtered off along with anything else to be rejected.

The baked residues were treated with 7 ml. of full-strength hydrochloric acid and washed from the dishes into Erlenmeyer flasks with water and 3 or 4 drops of nitric acid. Policing appeared to be unavoidable. The solutions were brought to boiling and saturated with hydrogen sulphide as they cooled. A pressure of a pound or so of the gas was maintained on the closed flasks for about an hour. According to Delage, the small quantity of nitric acid causes some sulphur to be precipitated and this serves to entrain and

carry down the copper sulphide. The precipitate was filtered into a Gooch crucible, which was then placed in a 250-ml. beaker. Two ml. of nitric acid were poured into the crucible and 3 ml. were used to rinse the flask and then poured into the beaker. The beaker was covered and heated on the hot plate until all the brown fumes and much of the nitric acid were gone. When the crucible had cooled it was filled with water and hung in a glass support against the side of the beaker until the asbestos mat had settled well into place. The contents of the beaker were filtered through the crucible back into the flask from which it had come, and then again transferred to the beaker and evaporated to dryness. The solution obtained by taking up this residue should contain all the copper.

The mineral constituents of the leaves were found to be inconsiderable in comparison with those of the spray deposit. The latter was known to contain calcium, phosphate, silicate, iron, lead, arsenic, magnesium, and aluminum, in addition to copper. Iron, calcium, and silica were expected to interfere with the determination of small quantities of copper. The steps just described have been recommended to remove these interfering substances. The silica should remain insoluble through the separation and be caught on the asbestos mat. The iron should pass through the first filtration and so be discarded. In the presence of phosphate or sulphate, however, some calcium remains undissolved during the first filtration, and the treatment with nitric acid dissolves some of it, so that this passes through the second filtration with the copper. If the solution be made alkaline with ammonia during the determination of the copper, this calcium will precipitate out and will have a bad effect on the result. In the work reported here it became the rule to add ammonia when the solution was being made up to 25 ml.; then, if a precipitate appeared, it was removed by centrifuging, relying upon the findings of Guillemet (11) that this procedure would not cause copper to be lost. There is still reason to be concerned over the separation of copper as the sulphide, because it has been found that significant quantities of copper can escape during this operation. In laboratories where only the smallest quantities of copper are determined, the extraction of the copper dithizone compound (16) or the carbamate compound (13) offers advantages.

Study of the results obtained by following the directions for the separation of copper as given by Delage (5) and by Haas and Quayle (12) suggested that the concentration of hydrochloric acid in the solution during saturation with hydrogen sulphide can be made less than is generally recommended. Haas and Quayle recommended about 10 per cent of gaseous hydrochloric acid (about 30 per cent of the reagent), and warned that no more than 15 per cent is permissible. Under the conditions of the work here, it was found that 10 per cent of the reagent (3 per cent of the sub-

stance) could be depended upon to prevent the precipitation of the lead, and that reduction of the acidity to this figure reduced the quantity of copper that escaped precipitation. The acetic acid-hydrogen sulphide wash solution was delivered from an elevated reservoir through tubing.

In 1935 3 different methods for the final determination of copper were exhaustively tested. The first of these was the "chromotropic" method of Ansbacher and Cherbuliez, as described by Ansbacher, Remington, and Culp (1). This method had been in use in this laboratory for 3 years, and in the course of that time a procedure practically identical with that described by Sheets, Pearson, and Geiger (15) was developed. The results of a statistical comparison of the series of determinations by this method with similar series by the other 2 on solutions of predetermined and of unknown copper content may well be omitted because the method has been dropped here for the following reasons: It has not been possible to provide a suitable, dependably uniform illumination for viewing the colors; the dry reagent, stored as received in a dark brown, glass-stoppered bottle, has undergone some obscure deterioration. Its strength apparently has undergone a progressive increase until after three years' preparation of the reagent according to the original directions yielded test solutions not less than 3 times as strong as they should be. The colors also have become progressively less distinctive and the end point more and more obscure. This opinion was confirmed by 2 others who had occasion to try this method in this laboratory.

The carbamate method, proposed by Callan and Henderson (3), has been widely used and it has been said that their reagent for copper—sodium diethyl dithiocarbamate—is probably the most sensitive known. Ansbacher, Remington, and Culp (1) objected to a turbidity that often appeared in these determinations. McFarlane (13) proposed to avoid this by an extraction of the colored substance with amyl alcohol and comparison or matching in that solution. Delage (5) proposed to add 2 drops of 1 per cent saponin solution in water to the determination and thus avoid precipitation of the colored compound. The latter course, being much less expensive, was followed here. Moreover, it was found advantageous to make up the final solution to 25 ml. and to include enough ammonia so that this solution was certainly alkaline. Then, if a precipitate formed, the entire contents of the flask was transferred to a centrifuge tube and portions for analysis were taken from the clear supernatant liquid after centrifuging. Solutions thus treated appeared to be very satisfactory matches for the standard.

The colored compound does show a tendency to agglomerate and settle out if there is more than a very little of it: hence, a determination that colors too deeply for matching usually will become turbid before it can be saved by dilution. If there is any likelihood that the first trial will contain more than 50 micrograms, it is well to arrange the work so that another por-

TABLE 1.—Total surface and internal copper found in leaves of pear trees treated with different spray mixtures on different dates, calculated in micrograms of copper per sq. cm. of total upper and lower leaf surface

Date	Remarks	Spray Treatments				
		No. 1	No. 2	No. 3	No. 4	Lime sulphur- Bordeaux mixture
		Copper phosphate mixture	No. 1 + aromatic sulphonate	No. 1 + synthetic resin mixture	No. 1 + fish-oil soap	
		Micrograms copper	Micrograms copper	Micrograms copper	Micrograms copper	Micrograms copper
1935						
	May 28	12.6	11.2	8.8	13.9	0.05
	May 31	10.9	11.8	9.6	9.8	0.15
	June 3	10.1	8.4	7.4	6.2	0.6
	June 6	14.6	13.0	11.4	13.6	0.18
	8	19.2	13.7	13.6	13.0	0.03
	10	16.7	11.9	10.8	12.0	0.21
	13	15.3	17.1	13.9	13.1	0.12
	15	19.7	16.7	9.9	9.3	0.3
	18	12.5	14.0	11.8	14.9	0.47
July	25	11.4	10.7	9.5	9.7	
	28	16.3	18.4	13.2	15.6	4.1
	3	19.2	15.7	13.7	13.5	4.5
	8	13.4	16.1	16.3	14.1	0.8
	10	11.9	18.5	16.2	13.7	1.2
	15	11.2	14.1	17.2	9.9	1.2
	23	8.4	5.7	5.8	10.8	0.65
	29	14.8	7.9	13.6	13.1	1.4
	Aug. 1	20.0	19.9	15.6	27.5	1.1
	8	18.1	16.5	12.3	20.7	3.7
Aug.	22	7.7	15.5	12.4	16.3	3.1
	26	7.5	11.7	6.8	11.1	0.4
	19	8.3	9.6	6.0	14.6	0.88
	1	3.4	9.2	8.9	10.4	1.9

<sup>a</sup> Duplicate sample.

Non-sprayed leaves were found to contain copper calculated on the above basis in the following amounts: 0.05, 0.17, 0.04, 0.12, 0.02, 0.06, 0.18 micrograms.

Note that the lime sulphur-Bordeaux mixture or standard-treatment plot received Bordeaux mixture on June 26 and July 31. The other applications were of lime-sulphur solution, and contained no copper.

From the average of duplicates as given above, the treatments rank, in respect to copper in the deposits, in the following order:

No. 1 is first 10 times, second 6 times, third 5 times, fourth 2 times.

No. 2 is first 6 times, second 10 times, third 4 times, fourth 3 times.

No. 3 is first 2 times, second 2 times, third 8 times, fourth 11 times.

No. 4 is first 4 times, second 5 times, third 6 times, fourth 7 times.



tion is available for a new determination that will contain less than 50 micrograms.

During the course of the work in 1935, the report of Haas and Quayle (12) on copper in citrus leaves came to hand. This report describes a method for the determination of small quantities of copper by a procedure exactly similar to the iodometric determination of full-scale samples (up to 400 mg. Cu), except that 1/500 normal sodium thiosulphate is used and no visible precipitate of cuprous iodide appears. In view of the precautions recommended in the literature for the purpose of precipitating all possible cuprous ions (7), the smooth and sure operation of the procedure described is remarkable, since as mentioned, no cuprous iodide becomes insoluble. This method was found to be very suitable for the determination of the copper of leaves that had received a copper spray. The Callan-Henderson method was used for leaf samples where no copper-containing spray material had been applied.

#### RESULTS

Table 1 shows the results obtained in 1935 and the attempts to find a ranking of the treatments in the order of the quantities of copper found. There is no obvious superiority of one adhesive over the others or over the control; so, apparently, within the errors of the experiment, the presence of the adjuvants did not affect the quantity of copper per unit area in the residues of the sprays.

The work was substantially repeated in 1936. One very objectionable feature of the figures obtained in 1935 is their lack of concordance. Certain improvements in the chemical technique failed to improve this agreement between duplicate samples, so the size of the sample was called into question. Haas and Quayle (12) used 500 leaves for each sample. In the study here reported the quantity of copper to be found in a much smaller sample was quite enough for determination as described. Also, the experiment as planned could not be carried out if so many leaves were used for each sample, because, for the number of samples projected, there would not be enough of the same sort of leaves desired as a sample. These were the oldest leaves on spurs 5 feet from the ground at the outside of the tree. Younger leaves would not have received all the sprays, and leaves from twigs and sprouts are difficult to date. It was decided to try 20 leaf disks for each sample in 1936, taken in duplicate and cut as nearly simultaneously and alike as possible.

Table 2 still shows discrepancy between duplicates, but the results unequivocally point out that the residues containing soap are more adherent than any of the others.

The following data and notes on control are offered in answer to the paramount question of the benefits to be obtained from these adjuvants.

TABLE 2.—Total surface and internal copper found in leaves of pear trees treated with different spray mixtures on different dates, calculated in micrograms of copper per sq. cm. of total upper and lower leaf surface

Date	Remarks	Spray treatments										Nonsprayed			
		No. 1 Copper phosphate mixture	No. 2 No. 1 + aro- matic sulphonate	No. 3 No. 1 + syn- thetic resin mixture	No. 4 No. 1 + fish- oil soap	No. 5 No. 1 + cot- tonseed oil	Standard lime sulphur- Bordeaux mixture	Micrograms copper							
		Micrograms copper	Micrograms copper	Micrograms copper	Micrograms copper	Micrograms copper	Micrograms copper								
May 18 21 28	Sprayed May 15	11.47	12.16 <sup>a</sup>	10.25	12.74	11.32	11.22	13.12	14.88	14.88	12.22	1.54	0.65	Micrograms copper	
		6.61	5.25	7.56	6.10	7.95	7.95	8.51	14.56	9.66	9.41	0.54	0.71		
		7.23	5.66	8.70	7.22	5.88	5.64	6.20	5.99	10.00	8.00	0.61	0.85		
June 29 10 12	Sprayed May 29	12.01	15.75	12.86	11.58	11.96	13.05	19.54	18.04	19.03	19.60	0.85	2.06	Micrograms copper	
		7.88	8.56	10.79	10.73	11.32	11.87	15.00	14.28	14.37	15.27	0.80	1.80		
		6.52	8.82	9.60	9.70	9.46	6.99	17.02	13.61	14.47	14.49	1.00	0.40		
July 2 8 17		6.54	6.82	9.13	7.65	9.33	10.50	12.13	12.72	6.62	13.29	0.60	1.00	Micrograms copper	
		6.72	7.74	8.38	8.72	9.00	8.86	14.83	11.50	11.37	14.75	1.05	1.45		
		5.77	6.71	8.54	8.14	7.07	7.08	13.39	10.64	12.36	10.64	0.50	0.40		
Aug. 8 14 24	Sprayed July 18	5.82	6.95	6.73	5.97	7.29	7.04	11.04	14.20	12.51	13.49	0.56	0.49	Micrograms copper	
		5.46	5.92	8.04	7.39	7.08	6.39	10.88	10.78	10.95	12.92	0.40	0.41		
		15.90	19.78	17.25	16.97	16.55	15.93	17.05	26.08	19.70	18.48	2.75	4.28		
Sept. 2 8 21		8.71	6.08	10.21	6.87	11.15	9.64	14.47	13.89	16.01	13.04	2.41	2.80	Micrograms copper	
		9.50	9.60	7.94	9.20	8.92	8.02	14.80	13.73	13.73	20.66	2.27	2.00		
		11.09	6.88	10.60	8.32	10.08	9.29	15.41	13.50	12.96	17.05	1.73	2.58		
Oct. 6		8.71	10.53	10.10	9.18	8.98	8.85	14.33	13.94	16.70	15.95	1.63	1.08	Micrograms copper	
		5.94	5.90	8.64	6.50	7.95	8.00	12.67	12.00	14.34	10.00	2.83	1.42		
		6.32	5.90	6.60	5.60	6.10	6.60	8.80	10.70	10.60	9.40	0.76	1.70		
		5.13	6.40	7.90	6.80	7.40	5.65	12.37	11.96	11.04	10.78	1.91	1.59	Micrograms copper	
		5.18	6.17	8.17	6.50	6.36	6.94	11.67	10.54	10.46	12.44	0.90	1.05		
		4.87	5.88	6.61	5.10	6.21	6.94	8.94	7.73	10.75	9.14	0.78	0.67		
		5.61	5.58	6.64	6.67	6.43	6.77	7.97	10.38	11.66	9.08	0.60	1.13	Micrograms copper	

<sup>a</sup> Duplicate sample.

Note that the lime sulphur-Bordeaux mixture or standard-treatment plot received no copper, except in the sprays of May 29 and July 18. One spray was omitted because of the dryness of the weather.

Table 3 gives the data on the effect of adding the adjuvants to copper phosphate mixture in the control of pear fruit spot during the 1935 and 1936 seasons. The data clearly indicate that the copper phosphate mixture, with and without the addition of the adjuvants, gave almost a perfect control in both seasons. This, while demonstrating the efficiency of the mixture without adjuvants, necessarily detracted from the experiment on the effect of the various materials added to enhance the sticking qualities. The results show, however, that under the conditions of this experiment, the adjuvants were neither useful nor detrimental in the control of the disease.

TABLE 3.—*Effect of adding various adjuvants to copper phosphate mixture<sup>a</sup> on the control of pear fruit spot caused by *Fabreaa maculata**

Treatment	1935 <sup>b</sup>			1936 <sup>c</sup>		
	Fruits counted	Fruits diseased	Diseased	Fruits counted	Fruits diseased	Diseased
	No.	No.	Per cent	No.	No.	Per cent
Copper phosphate mixture <sup>a</sup> .....	3031	5	0.16	3369	4	0.12
Copper phosphate mixture + fish-oil soap 1-100 .....	2292	6	0.26	4236	6	0.14
Copper phosphate mixture + 1/8-100 aromatic sulfonate .....	1652	4	0.24	5386	14	0.26
Copper phosphate mixture + 2-300 <sup>d</sup> synthetic resin mixture .....	.....	.....	.....	1490	6	0.40
Copper phosphate mixture + cottonseed oil 0.25 per cent .....	.....	.....	.....	1883	2	0.10
None .....	8646	2466	28.98	20652	3692	17.90

<sup>a</sup> 2 lb. copper phosphate, 4 lb. lime; 2 lb. bentonite, 50 gal. water.

<sup>b</sup> 6 applications (4 of these for leaf spot).

<sup>c</sup> 5 applications (3 of these for leaf spot).

<sup>d</sup> No count made in 1935.

<sup>e</sup> Not applied in 1935.

In no case was the addition of the adjuvants found to be deleterious to the physical properties of the copper phosphate mixture. Of the 4 adjuvants tested, no injury or physiological effect was observed from the use of the soluble fish-oil soap, synthetic-resin mixture, or from the aromatic sulfonate, in either 1935 or 1936. The cottonseed-oil mixture, used only in 1936, caused a yellowing of the older leaves on certain trees. The injury was unlike that familiar to us when copper is known to be responsible, and appeared to be correlated with very dry weather. It is possible that the affected trees were injured in some manner in the cold winter of 1935, and the heavy spray residues may have accelerated transpiration beyond the ability of the leaves to replenish the supply. Since the injury appeared

only on certain trees, the combination of cottonseed oil and copper phosphate may be considered harmless when used under normal conditions.

#### DISCUSSION

According to the appearance of the residues, those obtained from the sprays containing the soap and the aromatic sulfonate were better than the others. These residues were of even thickness and resisted abrasion due to leaves rubbing against each other; although, in general, leaves from the inside of a tree were more heavily coated than the exposed outside leaves. The spray containing the resin mixture left an even deposit, although apparently not so heavy as the two just mentioned; but it failed to withstand abrasion to the same extent. The residue from the treatment with cottonseed oil appeared at first glance to be the heaviest of all, but closer examination revealed that it was uneven, being made up of heavy blotches separated by lightly covered areas. This residue was comparable to those produced by the addition of the soap and the detergent in resistance to abrasion.

The data on the quantity of copper are pertinent both to the question of excessive run off at the time of application and to that of the ability of the residue to withstand abrasion during intervals following application. The quantities of each adjuvant to be used to secure the best effect had already been roughly ascertained in our preliminary experiments; hence the untoward effects of excessive quantities mentioned in the introduction were not encountered. The differences observed in the effects of the adjuvants on the quantity of residue in 1935 as compared with 1936 may have been due to a difference in the distribution of rainfall. The growing season of 1936 was drier than that of 1935, and, while the leaves may have rubbed each other just as much in 1936 as in 1935, the scouring effect of such rubbing is obviously more severe when water is present. The solvent action and impact effects of the rain itself are also absent.

The usual inspection of the fungicidal spray residues always suggests the questions: Are the materials of the spray mixture deposited in the original ratio? Is this ratio maintained during weathering? The results of our experiments do not answer these questions, but show that none of the adjuvants interfered with the laying down and adherence of the fungicide.

The control of this fungus by the unmodified copper phosphate mixture, even with the differences in the weather of the 2 years, evidently was too good to permit significant differences in control to appear in such an experiment as this. If any adjuvant had greatly enhanced the effectiveness of the unmodified spray, it could hardly have been perceived. In fact, nothing short of a very serious interference with the fungicidal properties of the copper phosphate, lime, and bentonite could have become manifest in the figures for control. It is suggested that a much more dilute foundation

mixture be used in such experiments. Actually, 4 times as much copper is present per unit volume of the copper phosphate sprays as of the Bordeaux mixture, which is known to be effective. The use of a marginally effective spray is interdicted in many experiments because, under conditions of unexpected heavy infection, adequate control cannot be maintained, and the final results may show almost no control.

The preceding paragraph is especially pertinent to the data on the control with cottonseed oil as adjuvant. Because of the excess of effectiveness over the requirements, the present data are not in conflict with those obtained in other experiments (unpublished data), in which the addition of cottonseed oil greatly decreased the fungicidal effectiveness of the copper phosphate mixture. The differences in control obtained in this paper are to be taken as measures of the unavoidable lack of perfection in the application of sprays rather than as indicating differences in the inherent effectiveness of the materials applied. The observed interference of cottonseed oil with fungicidal properties is ascribed to its forming a relatively impervious coating that encloses the particles of active material and hinders the diffusion of copper in solution from them to fungus spores.

The columns of data obtained by analyzing leaf samples from the lime sulphur-Bordeaux mixture plots, while not directly useful in determining the effect of the adjuvants, give useful reference information in regard to the whole experiment. Until the time the change to Bordeaux mixture was made these figures indicated the quantity of copper to be expected in samples to which no copper had been applied. After that, they indicate a quantity of copper that will accomplish a satisfactory control of the disease if in an effective form. It is pointed out that the quantity of copper in all the deposits from all the copper phosphate sprays was always greater than this. This emphasizes what has previously been said that probably more copper was applied than was necessary.

#### SUMMARY

Kieffer pear trees were sprayed with 5 treatments containing copper phosphate as the fungicidal ingredient. All were alike, except that a different adjuvant was added to each of 4. Samples of fixed areas of the sprayed leaves of all plots were taken at appropriate intervals from May to October and analyzed for total copper. For the determinations reported, a procedure recently described by Haas and Quayle (12), using two-thousandths normal sodium thiosulphate solution was found suitable and convenient. In 1935 the quantity of copper per unit area was neither increased nor decreased by any of the adjuvants within the rather large error of sampling. The results show that all the adjuvants tried increased the initial deposit of copper in 1936, and at least 2 measurably increased the adhesion.

The use of a fish-oil soap consistently gave the largest deposit of copper in the second year. The data on the control of pear leaf blight, for which the sprays were applied, were inconclusive, apparently because all the sprays applied were far more effective than necessary.

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# THE TOXIC DOSE OF MEALY-BUG WILT OF PINEAPPLE<sup>1</sup>

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Information on mealy bug, *Pseudococcus brevipes* (Ckll.), wilt with respect to the number of mealy bugs concerned is useful from both practical and theoretical aspects. From the practical standpoint such data are pertinent to control measures which aim at reducing populations to a point at which wilt does not occur. From the theoretical standpoint the data contribute to our knowledge of those diseases that are produced by the feeding of toxiniferous insects.

Previous studies have recorded the results of infesting pineapple plants with single gravid females and with 50 mealy bugs of mixed ages and sizes.<sup>2</sup> One, 5, 10, 20 and 40 subgravid mealy bugs per plant were used in another series of experiments<sup>3</sup> in which the feeding period was constant. In that experiment an attempt was made to determine by the use of large numbers of test plants whether wilt resulted from mass action of the colony or incidence of toxic individuals. The conclusion reached was that the most susceptible plant required a toxic dose greater than that provided by the feeding of one mealy bug. Further studies on the effect of varying numbers of mealy bugs are described herein with the object of determining the toxic dose of mealy bug wilt in different seasons and with varying periods of exposure of the plants to mealy bugs' feeding.

## EXPERIMENTAL PLOTS AND METHODS

All the experiments herein described were conducted in field plots. Each season's planting was of necessity made in a separate field and with the planting material available at the time. All the plantings were included within the area of the Pineapple Experiment Station farm.

Prior to the experimental infestations of mealy bugs, the plants were regularly sprayed with oil emulsions. Infestations were so made as to provide a graded series of doses<sup>4</sup> that were allowed to remain on the plants for varying periods of time. In all cases the mealy bugs used were selected as mid-size subgravid females from plants collected from a wilting field. Plants in approximately the same state of wilt were collected in order to have the source of the mealy bugs as nearly uniform as possible (see footnote 2).

<sup>1</sup> Published with the approval of the Director as Technical Paper No. 104, of the Pineapple Experiment Station, University of Hawaii, Honolulu.

<sup>2</sup> Carter, W. The pineapple mealy bug, *Pseudococcus brevipes*, and wilt of pineapples. *Phytopath.* 23: 207-242. 1933.

<sup>3</sup> Carter, W., and C. T. Schmidt. Mass action phenomena in mealy bug wilt. *Ann. Ent. Soc. Amer.* 28: 396-403. 1935.

<sup>4</sup> A "dose" being the number of mealy bugs applied to a plant at one time.

With the development of control measures, however, it is no longer possible to find heavy infestations of mealy bugs on young first-ratoon suckers, and collections have, of necessity, been made in abandoned second and third ratoons. The bugs were sorted and the mid-size individuals collected in Petri dishes. From the composite colony thus obtained usually comprising several thousand individuals, aliquots of the size called for were removed by suction into vials in which the mealy bugs were transported to the field. The method of compositing the mealy bugs after removal from the plants assures a thorough mixing of the individual colonies so that the aliquots removed for application to individual test plants represent thoroughly randomized samples of the whole population.

The purpose of selecting mid-size mealy bugs is twofold: one, to avoid the use of mealy bugs too small to stand the technique of transfer; the other, to avoid including gravid females that might promptly reproduce on the test plant. This latter purpose is not always accomplished because some females reproduce while abnormally small.

#### Experiment 1

Applications of 50 mealy bugs per plant were applied in various-sized aliquots over a period of 10 days. The plots were sprayed out 1 month after the doses were completed. Each plot contained 16 plants. In this experiment, 5 bugs were applied daily for 10 days; 10 bugs every other day for 5 applications; 25 bugs for 2 applications, applied 5 days apart. In addition, single applications of 50 mealy bugs, and single applications of doses equal in size to the aliquots used were also applied in 2 series. The first of these was infested the first day, and the second, the last day of infestation (Table 1). There is a considerable degree of uniformity between the mean percentages of wilt occurring with doses of the same order, in spite of some variation between plots. It also is seen that the plots infested with single doses of 25 bugs showed a mean percentage as high as those with aliquots and single doses of 50 bugs. This is an example of what frequently occurs, namely, that for any particular experiment, there is a dosage that gives approximately the maximum percentage of wilt; increasing the dose beyond that does not result in a proportional increase in wilt percentage.

#### Experiment 2

Applications of 50 mealy bugs per plant were applied in various sized aliquots over a period of 10 days. The plots were sprayed out 10 days after the doses were completed. This experiment, comprising 20 plants per plot, repeated the infestation technique of Experiment 1, except that the period for which the bugs were allowed to feed was shortened and the single doses equal in size to the aliquots used were omitted. It was anticipated that the





smaller doses used in Experiment 3, which was conducted in contiguous beds, would serve as checks on the aliquots used in Experiment 2. Unfortunately, the percentages of wilt occurring in Experiment 2 were so much greater than those that occurred with equivalent doses in Experiment 3 that the 2 sets of data cannot be compared. Complete data on this experiment are found in table 2.

TABLE 2.—*Wilt resulting from doses of 50 mealy bugs per plant applied in aliquots of different size. Experiment begun, February 6, 1934. Plants approximately 2½ months old when infested*

Plot No.	1	1a	2	2a	3	3a	4	4a	5	5a
Day	Number of mealy bugs applied to each plant									
1 .....	50	50	5	5						
2 .....			5	5	10	10				
3 .....			5	5						
4 .....			5	5	10	10				
5 .....			5	5			25	25		
6 .....			5	5	10	10				
7 .....			5	5						
8 .....			5	5	10	10				
9 .....			5	5						
10 .....			5	5	10	10	25	25	50	50
Percentage wilt .....	100	90	60	90	70	85	80	75	65	75
Mean .....	95		75		78		78		70	

When variation between plots is considered it is evident that mean percentages of plants wilting as a result of these applications, were remarkably and uniformly high.

### Experiment 3

In this experiment, involving 20 plants per plot, doses of 1, 5, 10, 25 and 50 mealy bugs were employed, with feeding periods of 1, 2, 5, 11, 15 and 20 days, and infestation periods from January 24, to February 15, 1934.

TABLE 3.—*Wilt resulting from doses of 1, 5, 10, 25 and 50 mealy bugs for feeding periods of 1, 2, 5, 11, 15 and 20 days. Plants approximately 2½ months old when infested*

No. of days	No. of mealy bugs					Infestation date
	1	5	10	25	50	
	Percentage of wilt					
20 .....	5	0	5	45	40	1/24/34
15 .....	0	0	5	10	10	1/24/34
11 .....	0	5	10	10	30	2/1/34
5 .....	0	5	15	0	15	2/1/34
2 .....	0	0	0	15	10	1/31/34
1 .....	0	0	0	5	0	2/1/34

Larger doses and longer feeding periods, both, seemed to cause higher percentages of wilted plants (Table 3). The highest percentage, however,

falls far short of those percentages obtained in Experiment 2, which was conducted in contiguous beds, planted with material of the same origin, and given similar agronomic treatment. Mealy bugs used in both experiments were taken from the same field, and the only known variable was the lapse of a few days between the last infestation in Experiment 3, and the beginning of infestations for Experiment 2.

It is seen from these data that 1 plant in the plot infested with 1 mealy bug per plant wilted. This occurred 3 months after infestation and the plant that wilted was much smaller than the surrounding plants. None of the plots showed as much as 50 per cent wilt, but the two 20-day plots with 25 and 50 bugs, respectively, approximated this figure. What is perhaps significant is the fact that these last plots showed a more rapid and complete collapse of the wilted plants than occurred in other plots.

#### Experiment 4

In this experiment, comprising a variable number of plants per plot, doses of 1, 5, 10, 25, 50 and 100 mealy bugs were employed, with feeding periods of 1, 5, 10, 20, 40 and 60 days and infestation periods from April 16 to June 19, 1935.

The plot arrangement in this experiment was varied slightly from that of Experiment 1 in that dosages and periods of infestation were not arranged in serial order. The purpose of this was to avoid any possible agronomic gradients. Sharp gradients did develop, however, as growth progressed, but in the immediate area of the experimental plots they did not appear to be significant. What probably was of more importance was the extreme lack of uniformity of growth, particularly in the early stages. This lack of uniformity expressed itself in differences in size and vigor of growth as well as in color abnormalities. The latter were all recorded just prior to infestation, but no correlation between them and later wilt was evident. Wilt resulting from these infestations is recorded in table 4.

TABLE 4.—*Wilt resulting from doses of 1, 5, 10, 25, 50 and 100 mealy bugs for feeding periods of 1, 5, 10, 20, 40 and 60 days. Plants approximately 4½ months old when infested*

No. of days	Mealy bugs						Infestation date
	1	5	10	25	50	100	
	Percentage of wilt						
1 .....	12	12	31	29	25	50	4/16/35
5 .....	4	8	12	29	50	58	4/19/35
10 .....	0	13	20	33	47	53	4/16/35
20 .....	0	0	5	11	17	55	4/24/35
40 .....	0	12	12	18	53	47	4/24/35
60 .....	0	10	10	30	40	70	4/20/35

It is most probable that the lack of uniformity in growth was associated also with variation in susceptibility as was indicated in Experiment 2.

Reference to table 4 will show that 12 per cent (2 out of 16 plants) infested with 1 mealy bug for 1 day wilted and that 4 per cent (1 out of 25) wilted when a single mealy-bug infestation was sprayed out after 5 days. This represents an extremely susceptible condition, which, fortunately, is not frequently met with in commercial fields.

When each day's infestations are considered it will be noted that with increasing numbers of mealy bugs used, the proportion of wilting plants increased, and, with few exceptions, a line drawn between the 25- and 50-bug doses will roughly separate the high percentages from the low. Again, it is observable that increased doses do not result in proportional increases in wilt percentage. This horizontal relationship in the table is obviously of more significance than the vertical, which shows the effect of length of time elapsing before the plants were sprayed out.

The presumption was made in this and previous experiments that differences in length of time elapsing between infesting and the completed spraying of the plants assure coordinate differences in the amount of feeding that takes place. In connection with the 2 following experiments, data were obtained on this point.

#### Experiment 5

In this experiment, consisting of 20 plants per plot, doses of 1, 5, 10, 25, 50 and 100 mealy bugs were employed for feeding periods of 1, 5, 10, 20, 40 and 60 days and infestation periods from January 29 to April 6, 1936. Unusually vigorous plants from an extremely high yielding field were planted; growth was uniform and no abnormalities were observable prior to infestation. For these reasons it was felt that differences in susceptibility due to variations in planting material would be reduced to a minimum.

It will be observed (Table 5) that there is no apparent relationship between length of feeding time and wilt. A feature of these results is the relatively high percentages of wilt following the smaller doses and the disproportionate rise in percentage when the dose was increased from 10 to 25 bugs. Increases to 50 and 100 bugs per dose made only minor differences in wilt percentages. The data may also be arranged to show the progressive increases in percentage of wilt, as the numbers of bugs increased as shown in table 6.

Inspection of the plots prior to spraying out showed that no ants were present and that there had been a rapid disappearance of mealy bugs from the plants. To obtain quantitative data on this, a series of infestations was made in contiguous beds.

TABLE 5.—*Wilt resulting from doses of 1, 5, 10, 25, 50 and 100 mealy bugs for feeding periods of 1, 5, 10, 20, 40 and 60 days. Plants approximately 3 months old when infested*

No. of bugs	Days						Infestation date
	1	5	10	20	40	60	
	Percentage of wilt						
1 .....	5	5	15	5	0	0	1/29/36
5 .....	25	15	20	15	15	15	1/29/36
10 .....	0	25	10	25	30	25	1/29/36
25 .....	60	70	65	50	40	70	1/29/36
50 .....	70	60	80	55	70	60	2/5/36
100 .....	70	85	75	75	90	85	1/30/36

TABLE 6.—*Summary of data from Experiment 5*

Total no. of bugs used .....	120	600	1200	3000	6000	12,000
No. of bugs per plant .....	1	5	10	25	50	100
Per cent wilt .....	5	17	19	59	66	80

One hundred twenty plants infested with 50 mealy bugs each, on Feb. 18, 1936, were dug up 2 days later, the plants dissected, and the mealy bugs counted. Establishment varied from 5 to 42 mealy bugs with an average of 26.53.

A second series then was set out in which varying numbers of mealy bugs were applied and left undisturbed for periods of 4 and 10 weeks before the plants were pulled and dissected and the mealy bugs removed and counted (Table 7).

It is clear from these data that the mealy bugs used in artificial infestations do not all become established, and that where ant colonies are not present the long periods of infestation are not significant, except in the case of the high initial mealy-bug numbers. In these last-named cases the periods elapsing prior to spraying out are long enough to permit reproduction of some of the individuals originally used. An interesting and perhaps significant point from these data is the comparison between the left and right sides of each bed, shown in its extreme case in Plot 5. In this plot it is seen that with the exception of 3 plants at one end of the right side, all the surviving populations were on the left side.

#### Experiment 6

In this experiment of 20 plants per plot, doses of 1, 5, 10, 25, 50 and 100 mealy bugs were maintained for feeding periods of 1, 5, 10 and 20 days and infestation periods from July 23 to August 12, 1936. In this, the last experiment of the series, the plants infested were in beds adjacent to those used in

TABLE 7.—Data from experiments to determine extent of establishment of mealy-bug colonies after application to pineapple plants. Plants dissected; all mealy bugs present, counted

Plot 1. Infested: 10 bugs 3/19/36; Plants 1-10: 10 additional 3/20/36; Counted: 4/15/36			Plot 2. Infested: 50 bugs 3/19/36; Counted: 4/15/36			Plot 3. Infested: 5 bugs 3/19/36; 5 bugs 3/20/36; Counted: 6/4/36			Plot 4. Infested: 5 bugs 3/20/36; Counted: 6/4/36			Plot 5. Infested: 50 bugs 3/20/36; Counted: 6/4/36			Plot 6. Infested: 10 bugs 3/20/36; Counted: 6/4/36			Plot 7. Infested: 50 bugs 3/20/36; Counted: 6/4/36		
Plant No.	Mealy bugs		Plant No.	Mealy bugs		Plant No.	Mealy bugs		Plant No.	Mealy bugs		Plant No.	Mealy bugs		Plant No.	Mealy bugs		Plant No.	Mealy bugs	
	Left	Right		Left	Right		Left	Right		Left	Right		Left	Right		Left	Right		Left	Right
1	0	0	1	1	1	1	0	0	1	0	0	1	0	0	1	49	0	1	31	0
2	0	0	2	0	0	2	0	0	2	0	0	2	0	0	2	0	0	2	0	0
3	0	0	3	3	1	3	0	0	3	0	0	3	0	0	3	29	0	3	0	0
4	1	0	4	4	5	4	0	0	4	0	0	4	0	0	4	0	0	4	2	1
5	0	0	5	1	0	5	0	0	5	0	0	5	0	0	5	0	0	5	2	0
6	0	0	6	0	0	6	0	0	6	0	0	6	0	0	6	3	0	6	0	0
7	0	0	7	0	0	7	0	0	7	0	0	7	0	0	7	10	0	7	0	0
8	0	0	8	0	0	8	0	0	8	0	0	8	0	0	8	0	0	8	0	0
9	0	1	9	0	0	9	0	0	9	0	0	9	0	0	9	0	0	9	0	1
10	5	0	10	0	0	10	0	0	10	0	0	10	0	0	10	0	0	10	2	2
11	1	0	11	0	0	11	0	0	11	0	0	11	0	0	11	4	0	11	0	0
12	0	0	12	0	0	12	0	0	12	0	0	12	0	0	12	0	0	12	0	0
13	0	0	13	0	0	13	0	0	13	0	0	13	0	0	13	0	0	13	3	0
14	0	0	14	0	0	14	0	0	14	0	0	14	0	0	14	62	0	14	0	0
15	0	0	15	0	0	15	0	0	15	0	0	15	0	0	15	36	0	15	0	0
16	3	0	16	0	0	16	0	0	16	0	0	16	0	0	16	15	0	16	2	1
17	2	0	17	0	0	17	0	0	17	0	0	17	0	0	17	11	0	17	1	1
18	0	0	18	0	0	18	0	0	18	0	0	18	0	0	18	5	0	18	0	0
19	0	1	19	0	0	19	0	0	19	0	0	19	0	0	19	19	0	19	0	0
20	1	0	20	0	0	20	0	0	20	0	0	20	0	0	20	24	0	20	4	0
21	1	0														1	0		5	0
22	0	0														0	0		8	0
23	0	0														0	0		4	0
24	0	0														0	0		5	0
25	0	0														0	0		0	0



Experiment 5, were of the same origin, and had had the same agronomic treatment. A comparison of the 2 experimental results was expected, therefore, to yield data on the effect of age of plant on the susceptibility to doses of varying size. All the plants were examined at intervals following infestation and all visible mealy bugs counted. Examples of these data are presented in table 8 and a summary of the wilt obtained is shown in table 9.

TABLE 9.—*Wilt resulting from doses of 1, 5, 10, 25, 50 and 100 mealy bugs for feeding periods of 1, 5, 10 and 20 days. Plants approximately 9 months old when infested*

No. of days	Mealy bugs						Infestation date
	1	5	10	25	50	100	
	Percentage of wilt						
1 .....	5	0	5	0	20	35	7/23/36
5 .....	0	5	5	5	15	40	7/24/36
10 .....	0	0	10	5	5	10	7/24/36
20 .....	0	5	5	0	20	a	7/23/36

<sup>a</sup> Not infested.

In this experiment occurred the expected reduction in susceptibility associated with older plants. This expressed itself in the reduced percentage of wilt and also in the increase of time elapsing between infestation and appearance of wilt symptoms. The 1 plant wilting after 1 bug had fed on it for 1 day represents a combination of insect toxicity and plant susceptibility that is most unusual for plants of this age.

#### DISCUSSION

It has been shown that under the artificial conditions of infestation necessarily employed, the number of mealy bugs applied to any one plant is quickly reduced, so that the doses of varying size serve only to provide actual infestations roughly proportionate, but not equal to, the original numbers applied.

For any given set of conditions, the percentage of wilting plants increases with increasing numbers of mealy bugs, but the ratio of wilt percentage to dose is not a constant. Scattered plants wilt after infestation by small doses of mealy bugs, but there usually is a disproportionate rise in wilt percentage at some point in the dosage scale. Increases in dose beyond this point are not accompanied by proportionate increases in wilt percentage. This is most clearly illustrated by tables 5 and 6. It is equally clear that no 2 experimental series, separated in point of time, yield the same percentages of wilt following similar doses of mealy bugs, a fact clearly disclosed by a comparison of tables 3 and 4.

It also is evident from the data that mealy-bug toxicity and plant susceptibility are both extremely variable. It is known (see footnote 2) that mealy bugs, from pineapples in various stages of growth, are variable in toxicity.



The technique used in collection of infested plants and the subsequent randomization of mealy-bug colonies is, however, such as to render this variable of little significance in any particular experimental series, although collections made on different dates and from different areas will unquestionably vary considerably.

Establishment and, therefore, amount of feeding, have been shown herein to vary within wide limits, but, again, in view of the technique of mealy-bug collection, this phenomenon is interpreted as largely a matter of plant suitability.

The variable of plant susceptibility, however, is without doubt a most important one. Even when apparently uniform planting material is used, there is considerable variation in growth and succulence. Moreover, the comparison between Experiments 2 and 3 suggests strongly that susceptibility may be due to a specific and perhaps fugitive state of the plant at the time of infestation.

Lastly, the possible contribution of a soil complex and its influence on the plant's susceptibility have been repeatedly suggested by the localized incidence of wilt in experimental plots. Diagrams illustrating this have been published in a recent paper.<sup>5</sup> The phenomenon is one that has occurred in greater or less degree in all field experiments. It is true that the localized establishment of mealy bugs, as shown in table 7, may well account for localization of wilt incidence, but the localized establishment of mealy bugs still remains an unexplained phenomenon of the microenvironment.

It is evident that further advances will be conditioned by the success achieved in attempts to standardize mealy-bug toxicity and plant susceptibility.

#### SUMMARY

A series of experiments on the relationship between numbers of mealy bugs applied to pineapple plants and resulting wilt, has been conducted.

Occasional plants wilt following infestation by single mealy bugs.

The percentage of pineapple plants wilting as a result of infestation by mealy-bug colonies of varying size increases with the number of mealy bugs. This increase is not directly proportional to the number of mealy bugs, there usually being a point on the dosage scale that brings a disproportionate rise in wilt percentage: increased dosages beyond this point result in only small increases in wilt percentage.

Variability between different experimental series is extremely high. The probable variables involved are discussed.

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<sup>5</sup> Carter, W. Insects and plant diseases. Hawaii. Ent. Soc. Proc. 9: 159-170. 1936.

## CONTROL OF DOWNY MILDEW OF TOBACCO BY VAPORS OF BENZOL AND OF OTHER ORGANIC SUBSTANCES<sup>1,2</sup>

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Recent investigations in Australia (1, 3, 6, 8) have demonstrated the efficacy of certain hydrocarbons, notably benzol, in the control of downy mildew of tobacco, caused by *Peronospora tabacina* Adam. This disease has long been very destructive in Australia, where many investigations have been conducted to find a satisfactory means for its prevention and control. The measures employed have included the artificial heating of beds in which the seedlings were being grown (5), the production of seedlings in areas remote from those in which the crop was being cultivated, and the application, as sprays or as dusts, of a variety of fungicidal materials (5). None of these control measures was found to have sufficient merit to warrant general adoption or to be regarded as a practical solution of the problem of downy-mildew control.

Similarly, within the United States, the application of various fungicides has consistently failed to give satisfactory control. Such an outcome is to be anticipated in the light of findings relative to the life history of the pathogen. These studies (2, 4, 9, 10) have established (a) that sporangia may be airborne; (b) that they may be produced daily, a new crop being formed each morning; (c) that infection from germinating sporangia may be accomplished within a brief period of time, and (d) that entrance of infection hyphae may be effected through both leaf surfaces. These facts become highly significant if coupled with the facts that the leaves of tobacco seedlings expand rapidly, and that it is almost impossible to secure complete coverage of their leaf surfaces with sprays or dusts. If the leaf surfaces could be entirely covered, moreover, the maintenance of adequate protection against infection would necessitate, as a minimum, the daily application of fungicides. These facts have inclined us, therefore, consistently to maintain, that the use of sprays or dusts against downy mildew of tobacco is not a rational procedure, and led us to postulate that adequate control might result if some

<sup>1</sup> Benzol is herein used instead of the more strictly correct term benzene, to designate the aromatic hydrocarbon,  $C_6H_6$ , in order to avoid confusion with benzine, which is a commercial mixture of aliphatic hydrocarbons, closely related to gasoline.

<sup>2</sup> A cooperative investigation by the Departments of Botany and Chemistry, Duke University. It is a pleasure to acknowledge our indebtedness to E. G. Moss and James Bullock, Tobacco Experimental Station, Oxford, N. C., for their cooperation; to L. F. Mandelson, Brisbane, Australia, for advice regarding experimental procedures, method of treating cotton fabrics, and progress of vapor treatments in Australia, and to E. G. Beinhart, Agricultural Adjustment Administration, Washington, D. C., for providing the covers used at Oxford during the past season.

gaseous fungicidal material were employed. To test this possibility, the writers, in 1934, employed ammonia vapors, obtained from the volatilization of ammonium carbonate, also chloropicrin, and diphenyl ether, in preliminary laboratory and field trials. In no case did the results with these substances give indication that they could be used effectively against downy mildew. This led us, in 1935, to laboratory tests of other substances, to a more systematic survey of volatile materials that might possibly be suitable, and to tests with certain of these materials in seed beds in 1936. Meanwhile, in Australia, in 1934, in preliminary trials under glass, benzol and toluol vapors were employed with success by Angell, Hill, and Allan (3). Our further efforts were encouraged by the quite satisfactory control of downy mildew of tobacco in seed beds secured with benzol vapors (1, 6, 7, 8) during the autumn of 1935, by investigators in Australia.

The experiments of the Australian investigators and their collaborators involved the employment of seed beds in widely separated parts of the country. They used beds of several types of construction, provided with various types of covers. They experimented upon the interval between successive exposures of the seedlings to the vapors, and varied the concentration of vapors by modifying the ratio of evaporating surface to the area of seed bed. It should be indicated that these experiments constitute the first to employ hydrocarbons as fungicides to growing crops. In a report of Angell, Hill, and Allan (3), furthermore, brief reference is made to the previous use of hydrocarbons in soil sterilization and in the preservation of wood from decay.

A summary of the results of our experiments with volatile products in downy-mildew control is presented at this time, (a) to record the fact that benzol is an efficient fungicide against downy mildew in this country, confirming the results with this substance in Australia; (b) to indicate the possibility of utilizing benzol and other volatile substances, notably mono-chlorobenzene, in plant-disease control, and (c) to stimulate investigation by others on improvement of methods of application of volatile fungicides to seed beds.

#### EXPERIMENTS IN 1936

Our first systematic experimentation, under out-of-doors conditions, with volatile chemical compounds was conducted at the Tobacco Experimental Station, Oxford, North Carolina, where the following substances were employed: aniline, B,B,dichloroethyl ether, benzol, bromobenzene, butyl acetate, mono-chlorobenzene, ethylene chlorohydrin, kerosene, p-dichlorobenzene, and commercial xylene. These compounds were selected because they cover a wide range of active organic groups, have approximately the proper volatility in relation to their toxicity, and are feasible to use because of their commercial availability and inexpensiveness.

Compartments, containing 1 square yard each, were constructed of boards, along the margins of 2 seed beds, each  $50 \times 5$  yards in dimensions (Fig. 1). The compartments were 3 yards apart, and the intervening sur-

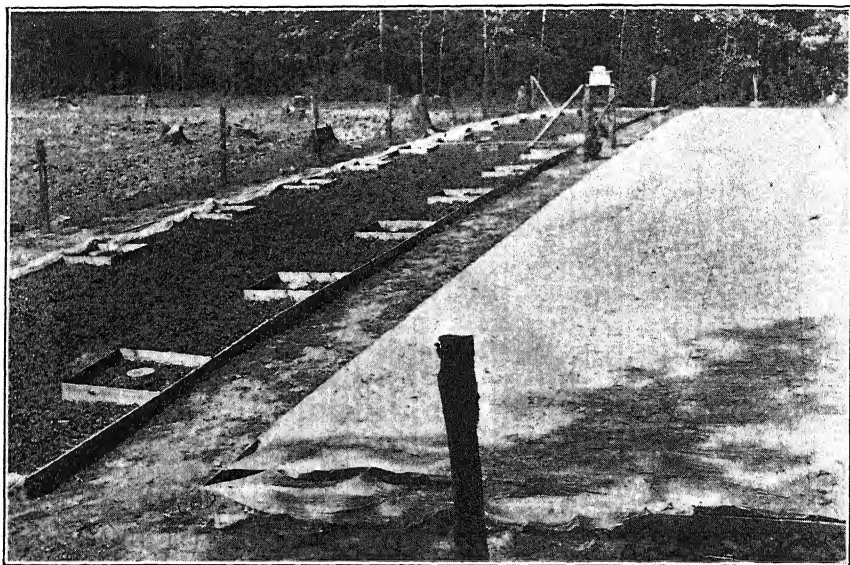


FIG. 1. Two seed beds, at Oxford, N. C., one covered and the other uncovered, used in trials with volatile substances, in 1936, to control downy mildew. Arrangement of compartments and evaporators is evident in the uncovered bed.

rounding seed-bed area was regarded as control. Six compartments were employed for each chemical. The compartments were covered with seed-bed cloth of the type usually employed for this purpose in the area devoted to the culture of flue-cured tobacco. Evaporators for the chemicals consisted of shallow pans over which a slightly larger pan was supported to function as a roof. An evaporator was placed near the center of each compartment. A continuous supply of chemical in the evaporators was maintained by frequent replenishment.

Treatment with volatile materials was initiated on April 20, 4 days prior to the outbreak of downy mildew, and was terminated on May 25. During this period temperature and moisture conditions were unfavorable for the development of the disease in serious proportions. This made it necessary artificially to inoculate the seedlings, and to water the beds and cover them with burlap so as to increase the relative humidity and lower the temperature. As a result the severity of infection was increased. Under these conditions kerosene, butyl acetate, and p-dichlorobenzene exhibited no apparent fungicidal value, nor did they cause injury to the plants. Although ethyl chlorohydrin was fungicidally effective at high concentrations,

it caused injury to the seedlings. At low concentrations it gave no control of the disease. Aniline, when undiluted, was lethal to the young plants. Benzol, B,B,dichloroethyl ether, and xylene each gave evidence of fungicidal value and of some toxicity to tobacco seedlings. Xylene was eliminated from further immediate trials by reason of its cost and of its structural similarity to benzol. B,B,dichloroethyl ether should be given further tests, as should aniline, using lower dilutions and modified conditions for evaporation. Mono-chlorobenzene completely inhibited the development of downy mildew and produced no toxic effects to the seedlings.

The knowledge gained from these experiments made it evident that among these substances, benzol and mono-chlorobenzene were most worthy of consideration in immediate future trials. It appeared that they might be used successfully provided it were possible to maintain the concentration of vapors within the seed beds, and, in the case of benzol, to retard the rate of volatilization. Experiments along these lines, in 1937, involved the use (a) of more tightly constructed seed beds covered with cloths that retarded the escape of the vapors, and (b) of benzol mixed with lubricating oil to retard the rate of evaporation of the benzol.

#### 1937 EXPERIMENTS

For the experiments, conducted near Lumberton, N. C., three compartments, 15 × 5 ft. in area, were constructed. In one, the fungicidal efficiency of benzol was tested, in another, of benzol mixed with lubricating oil<sup>3</sup> in the ratio of 1:5 by volume, and in the other of mono-chlorobenzene. This ratio of benzol to oil was chosen because it made possible the maintenance over a 12-14 hour period, of an average partial pressure of benzol vapor of approximately the same value as that of mono-chlorobenzene. The ratio was determined by preliminary laboratory trials of evaporation losses from various benzol-oil mixtures, in comparison with that from mono-chlorobenzene.

Five evaporators with a total evaporating surface equal to 1/54 of the area of the bed were placed in each compartment. An area of seed bed, 25 × 15 ft., situated at the end of these compartments served as a control (Fig. 2).

The cover for these beds was rolled back during daytime on fair days. It consisted of an inexpensive unbleached cotton sheeting of the following specifications: threads per inch, warp, 60, filling, 56; square yards per pound, 4. Beds were covered at night and on cloudy or rainy days. Before using, this sheeting was soaked for a day in a solution made by dissolving  $\frac{1}{2}$  pound of alum in 2 quarts of hot water, and then by diluting to  $2\frac{1}{2}$  gallons. After removal from this fluid, it was placed for 3 hours in another solution made

<sup>3</sup> The oil used was a cheap lubricating oil of light body. Its viscosity was 20 W, according to specifications of the American Society of Automotive Engineers. It would appear that waste oil drained from the crank cases of motors might be utilized instead.

by dissolving  $\frac{1}{4}$  pound of lead acetate in 2 quarts of boiling water, adding to this 1 ounce of glue-size that had been dissolved in water, and finally diluting to make  $2\frac{1}{2}$  gallons. The purpose of these treatments was to increase the vapor tightness of the fabric and to aid in preserving it against "mildew." The treatment proved to be ineffectual in waterproofing the cloth, since rains soon washed out the glue-size and water passed readily through the cover. This did not interfere, however, with the effectiveness of the cloth in retaining vapors. The reason for this appears to be that a film of moisture, from dew or rain, seals the interstices of the cloth and thus retards the passage outward of fungicidal vapors.

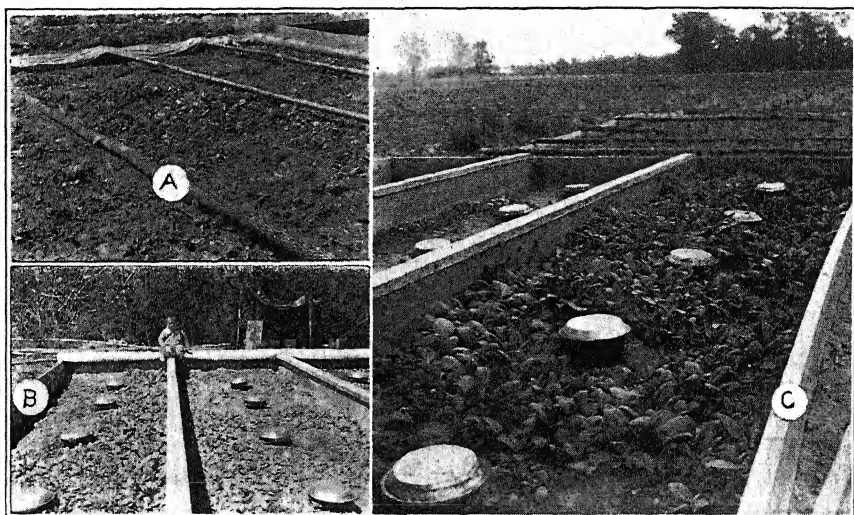


FIG. 2. Seed beds near Lumberton, N. C. A. Portion of nontreated seed bed a few days after complete invasion by *Peronospora tabacina*. B. At the left, compartment in which a benzol-oil mixture was applied; at the right, undiluted benzol. Compare with A and B photographed on March 27, 1937. C. In foreground, compartment in which seedlings were exposed to benzol vapor from benzol-oil mixture. In background, nontreated portion of seed bed with remaining plants still too small to be transplanted. Photographed on May 9, 1937.

Applications of volatile substances were begun March 19, about a week after primary infection was first apparent in near-by seed beds, and were continued until the season for transplanting was well advanced. The seedlings were exposed to vapors every night and during rainy days, necessitating replenishment of the chemicals every night and on rainy or cloudy mornings. Downy mildew became generally prevalent and unusually destructive in that region about 2 weeks after treatments were begun. Approximately 75 per cent of the seedlings in the nontreated portion of the bed were killed by the pathogen, whereas the disease remained entirely absent from the plants in

the 3 treated compartments. After recovery had begun in the control area there was a period of hot weather with high night temperatures. During this period, a lethal concentration of vapors was developed in the compartment in which undiluted benzol was applied. In consequence, most of the seedlings in this compartment were killed. No evidence of toxicity was found, however, among the seedlings treated with benzol-oil mixture. The plants subjected to this treatment, furthermore, were a deeper green than those in the other 2 compartments.

As a result of the perfect protection against downy mildew afforded in this experiment, it appeared desirable to institute further trials near Oxford, N. C., since the season at Oxford was approximately 3 weeks later than at Lumberton. Accordingly, 2 experiments were planned, one to be conducted on a grower's farm, and the other at the Experimental Station. In the first case, an area containing 54 square yards was segregated in which to apply the benzol-oil mixture. This experimental bed was situated along the margin of a bed containing approximately 500 square yards, and about 2 yards distant from the edge of another bed of equal area. These 2 areas of 950 square yards served as a control. Evaporating pans with an area equivalent to  $1/72$  of the seed-bed area were placed throughout the bed (Fig. 3). Benzol-oil mixture was placed in the evaporators each evening, and the beds remained covered during nights and on cloudy or rainy days. The cover was a nontreated cotton cloth with the following specifications: Threads per inch, warp, 68, filling, 72; square yards per pound, 3.25.

Fungicidal applications were begun April 22, and were discontinued on May 21. Infection was first evident in the treated area on April 22. It was first noted 3 days earlier, however, in the 950 square yards used as a control. By May 1, moderately severe infection involved the entire nontreated area, approximately half of the seedlings being killed. The efficacy of benzol vapor in downy-mildew control is shown in this test by the fact that none of the seedlings were killed by fungi in the treated area, and that no new lesions appeared after treatment was begun. The continuously healthy condition of the treated seedlings is indicated by the fact that on May 14, approximately 8000 were removed for transplanting. At this time none of the surviving seedlings in the nontreated area was of a size suitable for transplanting. By May 21, an additional lot of about 3000 seedlings from the treated bed were transplanted, making a total of 11,000 seedlings removed from the 54 square yards of treated seed-bed area. By this date only about 35,000 seedlings from the entire nontreated area of 950 square yards had developed to a size suitable for transplanting.

During the same period, 7 seed-bed areas ranging from 25 to 50 square yards each were used in the other experiment at Oxford. Four of these beds were treated with benzol-oil mixture. In 3, a 1:5 mixture of benzol and oil



was exposed in different types of evaporators, and in the other a 1:2.5 mixture. In one area, a 2:1 mixture of mono-chlorobenzene and oil was used, and the two remaining areas served as controls. Six of these beds were covered at night with a white fabric, of the kind used in the other experiment, just described, near Oxford. The other one was covered with an ordinary tobacco seed-bed cloth. This last bed was the one in which benzol was evaporated from the 1:2.5 benzol-oil mixture. The covers were removed during the daytime. At first a 1:72 ratio of evaporating area to seed-bed area was maintained in all beds. It soon became apparent, however, that in the bed covered with ordinary tobacco cloth, control of downy mildew was not being

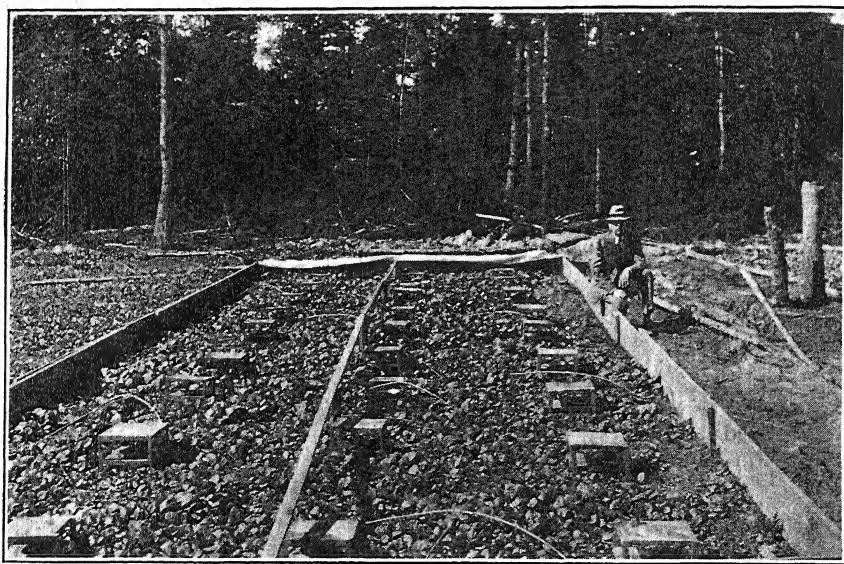


FIG. 3. Seed bed of grower near Oxford, N. C. Note vigorous condition of seedlings and distribution of evaporators. Contrast, in the upper left corner, the condition of the nontreated seedlings. Photographed on May 1.

accomplished; consequently in this case, the evaporating area was doubled and the benzol-oil mixture increased to a 1:1.25 ratio. Even this proved to be insufficient to give control, indicating that it is not possible to maintain a sufficiently high concentration of benzol vapor under ordinary tobacco cloth to be efficacious.

Applications of fungicides were begun on April 23, and discontinued May 24. Downy mildew was first apparent in the control beds on April 30, and the pathogen sporulated abundantly in them from May 4-18. Weather conditions were such, however, that the outbreak was only moderately severe.

It was apparent that the disease was continuing to spread and that control was not being accomplished in the bed in which the evaporators con-



tained a mixture of mono-chlorobenzene and oil. On May 10, therefore, pure mono-chlorobenzene was substituted for the mixture. Although a few sporangia developed in this bed subsequently, the disease was completely checked. This experience indicates that, even after downy mildew has become well-established, mono-chlorobenzene is effective in control.

The seedlings in one of the benzol-oil-mixture-treated beds remained entirely free from downy mildew. In this case the evaporators were so constructed that there was sufficient clearance between the cover and the part containing the volatile mixture. The seedlings in the other 2 beds treated with benzol-oil mixture bore a considerable number of lesions, but the pathogen did not kill any of the young plants, nor was it able to sporulate. As a result of comparative trials on the rates of evaporation from the different kinds of evaporators, it was found that the kinds used in these 2 beds did not allow a sufficiently rapid rate of evaporation. Undoubtedly, failure to secure complete control of downy mildew in these 2 beds was caused by the too low concentration of benzol vapors.

#### DISCUSSION

Our experiments have definitely confirmed those of Australian investigators in showing that satisfactory control of downy mildew of tobacco can be accomplished by the use of benzol vapor. It is apparent, furthermore, that a rather wide range exists between the concentration of benzol vapor that is effective against the downy-mildew organism and that that is toxic to tobacco seedlings.

The volatility of undiluted benzol would lead one to expect that, during hot weather, the benzol vapor could become sufficiently concentrated to be toxic or lethal to tobacco seedlings. In fact, injury from benzol vapor may occur at high temperature, as noted by Angell, Hill, and Allan (3) and substantiated by our own observations. It should be emphasized, however, that the vapor concentration from a benzol-oil mixture, in our experiments, completely prevented infection by *Peronospora tabacina* and produced no injurious effect upon the seedlings, whereas the vapor concentration from undiluted benzol, under identical environmental conditions, either produced serious injury or was lethal to tobacco seedlings. The use of benzol-oil mixture is preferable to that of undiluted benzol because the partial vapor pressure from the mixture is lower, and it is not possible, at ordinary temperatures, for a high vapor pressure to be developed from it. Of importance also is the fact that the rate of evaporation from the mixture is less than that from undiluted benzol, due to the higher viscosity of the mixture. By means of this mixture, a concentration of benzol vapor can be developed that completely inhibits infection by the downy mildew organism, and in addition, it can be maintained for long periods if the beds are covered with cloth of

sufficient tightness. This appropriate vapor concentration causes no ill effects upon the growth of tobacco seedlings. The use of a mixture of benzol and oil, therefore, provides an essential margin of safety and, as a consequence, renders benzol vapor treatments "fool proof," even under the wide range of environmental conditions that would be encountered in practice.

It appears to be neither necessary nor desirable to retard the rate of evaporation of mono-chlorobenzene when this substance is used in the control of tobacco downy mildew. This is due to the fact that mono-chlorobenzene is much less volatile than benzol.

Cotton fabrics of the quality of light-weight unbleached sheeting make satisfactory coverings for tobacco seed beds. Such cloths, if wet with dew or rain, are sufficiently vapor tight. They should not be waterproofed, since the seedlings require an abundance of moisture. It may prove to be advantageous, however, to treat seed-bed cloths to preserve them against deterioration by "mildews."

Several modifications of present day practices in seed-bed construction and handling should be made if benzol vapors or other volatile products are to be employed as a practical means of preventing or controlling tobacco downy mildew. These should include (a) the construction of long, narrow beds rather than wide, rectangular ones. This type of bed would facilitate the application of chemicals and obviate the necessity of trampling in the beds while attending the evaporators. (b) The seed bed should be tightly constructed. (c) Mechanical improvement in design of evaporators for the volatile materials should be made. If automatic devices are developed for this purpose their functioning should be dependent upon changes in concentration of fungicidal vapor in the air of the seed bed. The evaporators should be so constructed as to prevent the splashing out of fungicidal materials during rains and to permit easy replenishment. Kretchmar (7) in Australia, has devised an apparatus that facilitates rapid filling of the evaporators. (d) The covers should be so fitted that they may be easily removed on sunny days to permit the illumination necessary to the sturdy growth of tobacco seedlings.

The efficacy of substitutes for oil to retard evaporation of benzol should be given consideration in future investigations as should also the effectiveness of other volatile products. In this connection attention should be directed to Pittman's experiments (8) in which complete protection against downy mildew was secured, even in rainy weather, not only from benzol vapor but also from petrol and X3 solvent. The seedlings were completely destroyed in nearly all control beds, in these experiments. These results suggest that kerosene, typifying aliphatic hydrocarbon mixtures, should be given further trials.

The employment of fungicidal gases is a little-explored field of plant pathology. Conceivably volatile substances should have a wide field of use-

fulness in the control of diseases of crops grown under glass. Investigation of the efficacy of volatile substances would require, at the outset, a fund of information regarding physical and chemical properties of the substances to be tested, and a complete knowledge of the cycle of development of the specific pathogen. If, on the other hand, such a study were pursued by empirical methods, there is little, if any, likelihood that it could succeed.

#### SUMMARY

The vapor of benzol exerts a potent fungicidal effect upon *Peronospora tabacina*. This aromatic hydrocarbon has been demonstrated to be an efficient agency in the prevention and control of downy mildew of tobacco in seed beds. Mono-chlorobenzene can also be employed for the same purpose.

Benzol vapor in high concentration is toxic or lethal to tobacco seedlings. Lubricating oil, if mixed with benzol, retards the evaporation of benzol. An optimum concentration of benzol vapor can be secured by means of a mixture of benzol and lubricating oil. This optimal vapor concentration is neither toxic nor lethal to tobacco seedlings, regardless of weather conditions, but is fungicidally effective against tobacco downy mildew.

In vapor treatments, properly constructed beds should be employed. They should be covered during treatment with a cotton fabric of the texture of sheeting. The evaporators should have a surface approximating one seventy-second of that of the seed bed and have sufficient clearance to allow free evaporation.

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# NEGATIVE CORRELATION BETWEEN THE OCCURRENCE OF POLYPHENOL OXIDASE AND DIASTASE AND THE DEGREE OF INCIDENCE OF "BLACKHEART" OF POTATO

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Although the physiological disease of potato tubers, commonly known as "blackheart," has undoubtedly been a cause of considerable loss for many years, there appears to be no agreement among the various investigators with regard to the cause or causes of the disease. It was shown by Bartholomew (2) that it could be artificially induced by subjecting the tubers to a temperature of 38°-48° C. for 14-48 hours. Later, Bartholomew (3) explained the occurrence of the disease on the assumption that the asphyxiated tissues of the tubers develop an increased amount of the aromatic aminoacid tyrosine, which is acted upon by the enzyme tyrosinase, leading ultimately to the formation of melanins. He supposed that the action of tyrosinase was accelerated by access to an unusual amount of oxygen, due to the killing of the tuber cells. It was discovered accidentally by Stewart and Mix (9) that "by excluding the air from potatoes blackheart may be produced at temperatures much lower than those employed by Bartholomew." According to Davis (4), there is an accumulation of CO<sub>2</sub> and a depletion of oxygen in the tuber tissues prior to the appearance of the disease.

In the course of investigations on the oxidase and diastatic activities of stored potatoes, it was observed that the enzyme samples obtained from diseased potatoes were invariably of a lower degree of purity than those obtained from sound tubers. This led us to a thorough investigation of this aspect of the problem, and the data obtained in this connection are here presented.

## METHODS

The polyphenol oxidase was obtained in accordance with Szent-Gyorgyi's method (6). The activity of the enzyme was measured with the manometer devised by Singh and Mathur (7). The right-hand vessel of the manometer received 12-20 mg. oxidase preparation in 2.7 ml. phosphate buffer (pH 7.3) in its main part, while, in the tube attached to the bottom of the vessel, was put 0.5 ml. of 10 per cent KOH. The side tube contained 0.4 ml. of 2 per cent catechol solution. The left-hand vessel served to compensate the fluctuations in the temperature of the water bath, which was maintained at  $27 \pm 0.2^\circ \text{C}$ .

The iodine method was employed for the determination of diastatic activity. One ml. of 0.75 per cent soluble starch solution was placed in each

of 20 test tubes surrounded by ice. Increasing amounts of the diastase extract were added to the tubes beginning with 0.9 ml. and increasing the amount by 0.05 ml. for each succeeding tube, using toluene as a preservative. The tubes were then incubated at 35–36° C. for 36 hours, placed again in ice water, filled nearly full with water, and 2 drops of iodine solution were added to each tube. The first tube in the descending series, which showed a slight blue color, was considered the index for comparing diastatic activity.

The degree of incidence of the disease was determined quantitatively as follows: The potatoes were stored in hermetically sealed jars, maintained at different temperatures for a number of days. At the expiration of this period, the jars were opened and the tubers cut and examined for internal necrosis. It was observed that the diseased tubers ranged from perfectly sound ones to those having as much as 30 per cent of the cut surface discolored. When examining a potato the cut was always made in such a position and in such a direction as to expose a maximum diseased area. This necessitated under certain conditions the cutting of tubers twice or even 3 times. After the diseased area had been exposed, an outline was made in India ink, limiting the discolored area, as, also, the whole surface exposed by cutting the potato. The cut tuber was then lightly pressed against a sheet of mm.-squared paper and the area covered by the diseased portion, as well as the area of the total cut surface determined. The diseased area, when expressed as the percentage of the total area of the cut surface, gave an approximate measure of the degree of incidence of the disease.

The Haldane gas-analysis apparatus as adapted by Singh and Mathur (8) was employed in determining the composition of the atmosphere surrounding the tubers. A long glass tube was placed with one end terminating in the center of each basket of potatoes; through this tube air was withdrawn intermittently from the interior for analysis of CO<sub>2</sub> and oxygen content. The temperature in the interior of the basket of potatoes was recorded by means of an electrical resistance thermometer of a type usually employed for the measurement of soil temperatures.

#### RESULTS

Before studying the quantitative relation between oxidase and diastatic activities on the one hand and the degree of incidence of blackheart on the other, some preliminary experiments were performed. The experimental tubers were stored in large cylindrical baskets in a storage room. Out of potatoes that had been in storage for about 3½ months a quantity of tubers was sampled, taking care to select, as far as possible, potatoes of similar size and shape. They were then cut in two by means of a sharp scalpel and sorted into two lots, one of sound, the other of diseased tubers. These two lots were used subsequently for the determination of oxidase and diastatic

activities. Data pertaining to polyphenol oxidase activity are recorded in table 1. Although there are numerous variations in the activity of the enzyme preparations obtained from different lots of tubers and often the

TABLE 1.—*Activity of oxidase preparations obtained from sound and diseased tubers*

Oxidase	Time	Oxygen uptake c. mm. (N. T. P.)			
		Sound tubers		Diseased tubers	
		A	B	A	B
<i>mg.</i>	<i>min.</i>				
20.0 .....	20	179.3	180.3	140.9	138.3
15.0 .....	20	130.7	133.4	107.9	108.6
15.6 .....	20	127.2	129.2	109.2	108.7
15.5 .....	20	130.9	131.7	106.7	.....
15.7 .....	25	150.9	149.3	117.1	.....
14.8 .....	25	152.0	149.9	119.0	120.7
12.5 .....	25	142.7	140.7	103.7	102.9
12.6 .....	30	145.6	143.2	109.3	108.6
15.0 .....	30	.....	168.9	130.6	129.7
15.6 .....	30	167.7	168.2	133.6	130.2
15.8 .....	32	166.2	169.7	137.6	138.9
16.3 .....	32	170.6	171.2	140.3	141.2

duplicates differ widely, in general, the samples obtained from sound tubers show higher polyphenol oxidase activity than those obtained from diseased potatoes. Essentially similar data were obtained with regard to the diastatic activity. An examination of table 2 reveals the need of greater volumes of diastase extract for the hydrolysis of 1 ml. of 0.75 per cent soluble starch solution in the case of samples obtained from diseased tubers in comparison with those obtained from sound potatoes.

TABLE 2.—*Activity of diastase extracts obtained from sound and diseased tubers*

Quantities in ml. of diastase extract equivalent to 1 ml. starch solution			
Sound tubers		Diseased tubers	
A	B	A	B
1.20	1.10	1.25	1.25
1.30	1.30	1.30	1.30
1.30	1.30	1.35	1.30
1.00	1.00	1.00	1.00
1.10	1.00	1.25	1.25
1.65	1.65	1.65	1.65
1.35	1.35	1.45	1.45
1.50	1.55	1.55	1.60
1.40	1.35	1.50	1.50
1.15	1.15	1.20	1.20
1.40	1.45	1.20	1.20
1.15	1.10	1.10	1.15
1.10	1.10	1.05	1.05
1.05	1.05	1.00	1.00
0.95	0.95	1.05	1.10

Having satisfied ourselves that the diseased potatoes were characterized by a lowering of polyphenol oxidase and diastatic activities, we decided to elucidate quantitatively the relation between enzymic activity and the degree of incidence of the disease. To this end the disease was artificially induced in several lots of potatoes by enclosing them in hermetically sealed jars, maintained at a temperature of 48–49° C. for 4 days. At the expiration of this period parallel determinations of the degree of incidence of the disease on the one hand, and oxidase or diastatic activity on the other, were made. Relevant data are presented in tables 3 and 4. As indicated in table

TABLE 3.—*Degree of incidence of disease in relation to oxidase activity*

Discolored area	Oxidase activity		
	Time	Oxidase	Oxygen uptake
<i>Per cent</i>	<i>min.</i>	<i>mg.</i>	<i>c. mm.</i>
1.8 .....	20	12.7	165.2
2.1 .....	20	12.9	161.7
2.7 .....	20	10.2	159.2
2.2 .....	20	10.7	156.2
4.3 .....	20	11.7	150.7
4.3 .....	20	12.7	145.7
4.7 .....	25	11.2	140.8
5.9 .....	20	10.9	135.1
7.2 .....	20	11.7	129.2
9.1 .....	15	12.8	122.7
9.3 .....	20	11.3	115.9
13.3 .....	25	11.7	110.7
14.6 .....	20	12.1	97.2
19.7 .....	15	11.7	89.2
21.2 .....	20	11.2	80.7
27.3 .....	25	10.7	75.7
27.4 .....	25	11.6	65.7
27.4 .....	15	11.9	60.2
29.2 .....	20	12.1	58.7
29.7 .....	20	12.0	55.7

3, the various oxidase preparations are by no means of the same degree of purity, and equal weights of oxidase preparation rarely catalyze the oxidation of catechol to the same extent. As a matter of fact, the degree of purification<sup>1</sup> of the various enzyme preparations was by no means high, though it was of the same order in almost all samples, so that the data obtained are comparable. Generally speaking, purer samples of the polyphenol oxidase were obtained from potatoes that were slightly diseased than those obtained from tubers in which the disease was fairly advanced. In other words, decreasing percentage contents of polyphenol oxidase are associated with increasing degrees of incidence of the disease. Determinations of diastase activity (Table 4) gave essentially similar results. Here, also the duplicate

<sup>1</sup> The degree of purification of an enzyme is generally denoted by a number that is the ratio of enzymic activity per mg. dry weight of final preparation to the enzymic activity per mg. dry weight of the original material (5).

TABLE 4.—*Degree of incidence of disease in relation to diastatic activity*

Discolored area	ml. diastase extract equivalent to 1 ml. starch solution	
	A	B
<i>Per cent</i>		
2.9 .....	0.95	1.00
3.6 .....	0.95	1.00
3.9 .....	1.05	1.00
6.2 .....	1.10	.....
9.7 .....	1.35	.....
10.5 .....	1.40	1.45
11.2 .....	1.70	1.70
12.7 .....	1.85	1.85
12.7 .....	1.90	1.95
12.8 .....	1.90	1.80
17.2 .....	1.95	1.95
19.3 .....	2.00	1.80
21.7 .....	2.10	1.70
21.2 .....	2.30	1.95
22.0 .....	2.45	2.40
23.7 .....	2.45	2.45
24.6 .....	2.60	2.55
26.3 .....	2.70	2.75
27.3 .....	2.75	2.80

samples vary in their capacity to hydrolyze starch; but, in general, diastase extracts of a very low starch-hydrolyzing power were obtained from tubers in which the disease was fairly advanced.

This partial destruction of the enzymes in the diseased tubers indicated that probably a high temperature contributed mainly to the incidence of the disease. This point was investigated in the following manner. Potatoes were enclosed in 16 containers, 2 containers being placed at each of the temperatures of 7, 14, 21, 30, 37, 40, 47, 52 and 56 degrees C. for 6 days. At the expiration of this period the enzymic activity and the degree of disease incidence were determined. An examination of table 5 indicates that at the

TABLE 5.—*Effect of temperature on the incidence of blackheart, oxidase, and diastase activities*

Temp.	Diseased tubers	Oxidase activity <sup>a</sup>	Diastatic activity <sup>b</sup>
<i>°C.</i>	<i>Per cent</i>		
7 ± 0.4 .....	0.0	180.2	1.35
14 ± 0.2 .....	0.0	182.7	1.25
21 ± 0.1 .....	0.2	173.6	1.25
30 ± 0.2 .....	3.8	167.2	1.35
37 ± 0.2 .....	11.2	150.6	1.50
40 ± 0.3 .....	12.4	127.3	1.60
47 ± 0.3 .....	15.6	122.0	1.75
52 ± 0.4 .....	18.2	110.3	1.75
56 ± 0.5 .....	19.4	100.7	1.80

<sup>a</sup> Oxygen uptake in c.mm. (N.T.P.) of 12.5 mg. oxidase preparation in 25 minutes.

<sup>b</sup> ml. diastase extract equivalent to 1 ml. starch solution.



TABLE 6.—*Composition of the atmosphere surrounding the tubers and the temperature inside and outside the basket of potatoes during the storage period of 1935<sup>a</sup>*

Item	At time of storage <sup>b</sup>	15-day periods after storage										
		1	2	3	4	5	6	7	8	9	10	11
Carbon dioxide per cent .....	0.04	0.07	0.08	0.09	0.11	0.12	0.12	0.14	0.17	0.19	0.39	0.42
Oxygen per cent .....	20.8	20.0	19.9	19.7	19.2	19.1	19.1	19.0	18.8	18.6	18.5	17.9
Temperature, °C. (inside) .....	26.2	29.7	30.2	33.7	35.6	40.2	43.2	44.7	45.9	49.2	50.0	48.3
Temperature, °C. (outside) .....	26.0	28.9	29.2	32.0	33.0	37.1	39.2	40.2	41.7	44.2	45.0	43.3

<sup>a</sup> All figures given above represent averages of 10 determinations.

<sup>b</sup> Tubers were stored on Feb. 2, 1935.

temperatures of 7° and 14° C., the tubers remained perfectly sound, even after 6 days' enclosure in air-tight containers. This finding is significant in view of the fact that, when packing the tubers, every effort was made to leave the least gas space inside the containers. At a temperature of 21° C., only 0.2 per cent of the tubers showed visible signs of disease. At all these temperatures the enzyme preparations obtained were of a fairly high degree of purity. Another point brought out by the data is that the percentage of diseased tubers increases with an increase in the temperature at which the tubers are stored for inducing the disease. Moreover, the percentage concentration of both the polyphenol oxidase and the diastase decreases with increase in the percentage of diseased tubers.

The last series of experiments was conducted to investigate the increase in the temperature of the interior of the basket of potatoes during the storage period; and the extent to which the composition of the atmosphere surrounding the tubers undergoes variations during summer storage. The data (Table 6) indicate an accumulation of CO<sub>2</sub> and a depletion of oxygen in the atmosphere surrounding the tubers and progressive increases in percentage of CO<sub>2</sub> with increasing periods in storage. On the day of storage, the temperatures within and outside of the basket of potatoes are nearly the same, but the difference between the two gradually increases, until, in May and June, the inside temperature is about 5° higher than that outside.

#### DISCUSSION

A negative correlation between the polyphenol oxidase activity and the degree of incidence of blackheart appears significant; particularly, in view of the fact that the disease usually has been ascribed to some derangement in the respiratory process of the tubers. Whether or not our data confirm this view, depends upon the extent to which the concentration of the polyphenol oxidase on the one hand and the respiration intensity of the tubers on the other are related.

In an attempt to explain the mechanism of respiration, the oxidases generally have been assigned a rôle in the process, though, thus far, no direct evidence has been recorded to justify one's assuming such relationship. It is, however, difficult to assign a function to the oxidases, since, by the present known methods of measuring their activity, they have no direct action either upon the substances consumed in respiration or upon its various decomposition products. Appleman (1) found no correlation between oxidase activity and the rate of respiration in potato tubers. He stated that, while oxidases may play some rôle in the process, they are certainly not the controlling agents in regulating the rate of respiration. Keilin (6), on the other hand, found that all factors that inhibit activity of the oxidase, or destroy it completely, affect in the same way the oxygen uptake of cells. He came to the conclusion that "oxidase in the living, actively respiring cell represents

a link in the chain forming the complicated respiratory mechanism, in which several other respiratory ferments and systems are involved and intimately interconnected."

Our data indicate that sufficient heat is given off due to the respiration of potatoes to raise the temperature of the air in the interior of the basket about 5° C. above that prevailing in the storage room in the months of May and June. This increase in temperature may increase the rate of respiration of the tubers. Likewise, the data pertaining to the composition of the atmosphere surrounding the tubers indicate an accumulation of CO<sub>2</sub> and a depletion of oxygen in the surrounding air with increasing periods in storage.

Our findings when considered in conjunction with the data obtained by Davis (4), to the effect that there is an accumulation of CO<sub>2</sub> and a depletion of oxygen in the tuber tissues prior to the appearance of the disease, suggest that the disease is probably attributable to a derangement in the respiratory process of the tubers brought about by an increased temperature, and an accumulation of CO<sub>2</sub> and depletion of oxygen in the air surrounding the tubers.

It is a general observation that the appearance of the necessary enzymes in biological situations is coincident as to time and place with the occurrence of its substrate and reaction products. A lowered diastase activity may result in a lessened percentage of maltose in stored potatoes, which, in all probability, is hydrolyzed to yield the hexose substrate for respiration.

It appears probable that the incidence of the disease is referable to a partial destruction of polyphenol oxidase and diastase due to the heating of the tubers during summer storage accompanied by an accumulation of CO<sub>2</sub> and a depletion of oxygen in the atmosphere surrounding the tubers.

#### SUMMARY

Data pertaining to the polyphenol oxidase and diastatic activities obtained in the course of this investigation indicate a negative correlation between enzymic activity and the degree of incidence of the disease.

During the storage of potatoes in summer, sufficient heat is given off through tuber respiration to raise the temperature about 5° C. within the basket of potatoes above that prevailing in the storage room. Data concerning the composition of the atmosphere surrounding the tubers indicate an accumulation of CO<sub>2</sub> and a corresponding depletion of oxygen in the surrounding air.

It is suggested that a partial destruction of the enzymes due to the heating of the tubers during summer storage is probably responsible for the appearance of the disease.

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## TWO SEPTORIA LEAF-SPOT DISEASES OF RUBUS IN THE UNITED STATES<sup>1</sup>

(Accepted for publication July 15, 1937)

S. M. ZELLER

Darrow<sup>2</sup> has recently published on the susceptibility of *Rubus* to leaf spot at Beltsville, Maryland. The data presented there are not in agreement with those we have recorded for the relative susceptibility of certain varieties and species of *Rubus* when grown in the Pacific Northwest. For instance, the Lloyd George red raspberry never develops an appreciable amount of leaf spot when grown in localities in the Pacific Northwest where susceptible varieties are seriously damaged. On the other hand, it is reported this variety cannot be grown "commercially so far south as Maryland because it is too susceptible to leaf spot to produce even a small crop."

Upon request, G. M. Darrow has sent numerous collections of leaf spot from Maryland and from Willard and Whiteville, North Carolina, and F. A. Wolf has sent materials from Durham, North Carolina. These have been studied mycologically and compared with *Septoria* leaf spot on Himalaya blackberry kindly sent by Ralph V. Harris from East Malling, Kent, England. This has given ample material for a comparative study of the imperfect stages of the fungi associated with leaf spots collected in various localities.

A cursory examination of the material indicated that the *Septoria* leaf spots from the Pacific Northwest, Wisconsin, Durham, North Carolina, and England are essentially alike, and constitute the universal concept of *Septoria rubi* Westendorp. The leaf-spot material sent from Beltsville,

<sup>1</sup> Published as Technical Paper No. 264 with the approval of the Director of the Oregon Agricultural Experiment Station. Contribution from the Department of Botany.

<sup>2</sup> Darrow, G. M. Susceptibility of raspberry species and varieties to leaf spot (*Mycosphaerella rubi*) at Beltsville, Maryland. Phytopath. 25: 961-962. 1935.

Maryland, and Willard, North Carolina, however, has a different appearance. Leaves of the Young dewberry from Whiteville, North Carolina, are infected with both types of spot.

This variation in appearance of the spots suggested the presence of two different *Septorias*, and published accounts dealing with *Septoria* on *Rubus* were examined critically with this concept in mind. The Beltsville material proved to be a fungus first described by Saccardo<sup>3</sup> as *Septoria rubi* West. var. *brevispora* Sacc. from material on leaves of *Rubus hispidus* collected by C. H. Peck at North Chatham, New York. Through the courtesy of H. D. House, New York State Botanist, the type of this variety of *S. rubi* was studied and found to agree in all respects with the fungus and leaf spot so prevalent at Beltsville.

Fortunately, some leaves of *Rubus hispidus* in the type collection of *S. rubi* var. *brevispora* were infected with *S. rubi*. The difference in the leaf spots caused by the 2 fungi on the same host are illustrated in figure 1.

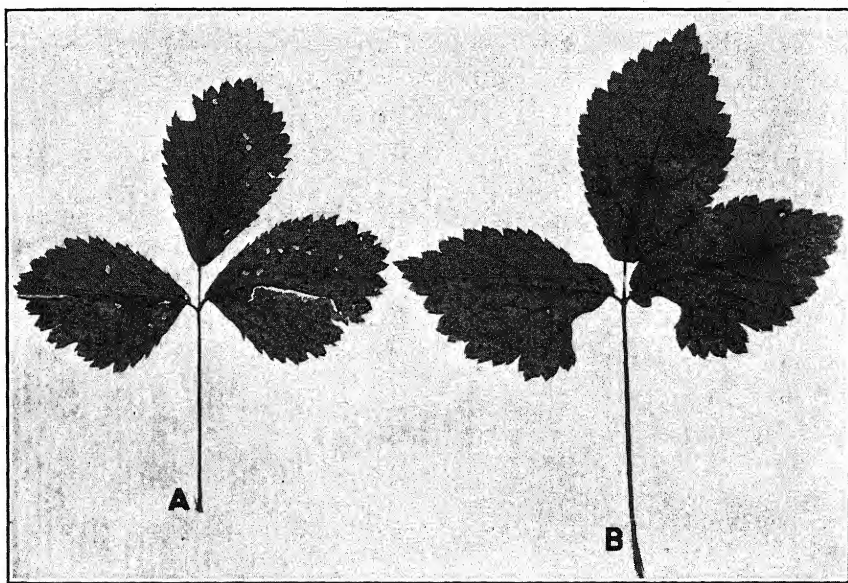


FIG. 1. Two leaves of *Rubus hispidus* in the type collection of *Septoria brevispora*. A. Leaf spot caused by *S. brevispora*. B. Leaf spot resulting from infection by *S. rubi*.

There are so many distinct physiological and morphological differences between the 2 fungi that the one cannot be considered a variety of the other. A new combination, therefore, is proposed raising the variety to specific rank, i.e., *Septoria brevispora* (Sacc.) n. comb. (Syn. *S. rubi* West. var. *brevispora* Saccardo).

<sup>3</sup> Saccardo, P. A. Notae Mycologicae, 20, series Nuovo Gior. Bot. Ital. 23: 185-234. 1916. (see p. 196.) Sylloge fungorum, v. 25. abellini. 1931. (see p. 447.)

*Septoria brevispora* (Sacc.) Zeller is described as follows:

Spots angular, following the veinlets in the leaf mesophyll, 1-2 mm. broad, zonate; cinnamon brown near the margin, light pinkish cinnamon within, turning light brownish gray, usually *not* surrounded by a purplish or reddish brown zone in the neighboring leaf tissue, as in the case of *S. rubi*; summer pycnidia epiphyllous, 5-18 to the spot (average 10.4), brownish, 46-60  $\mu$ . broad  $\times$  27-35  $\mu$ . high, thin-walled, with spores arising from base only; ostiole broad with no rostrate tendency; spores cylindric, obtuse at both ends, hyaline, 1-3 septate, 15-30 (36)  $\times$  1.8-3.4  $\mu$ .

*Septoria rubi* West. may be described as follows:

Spots rounded to angular, 0.5-1.5 mm. broad; brownish red, then whitish with purple border in the surrounding leaf tissue; summer pycnidia epiphyllous, 80-100  $\mu$ . broad  $\times$  75-85  $\mu$ . high, brown-black, oblate depressed, sides bulging, usually few, 1-7 to the spot (average 2.2), walls thin (*winter pycnidia* have heavy, dark walls, 3-4 cells thick, ostiole with no rostrate tendency, on stromata mostly above the leaf surface), with spores arising from base and sides; ostiole rather narrow, with somewhat of a rostrate tendency; spores filiform, 1-pluriseptate, rather pointed above, obtuse below, hyaline, 20-55  $\times$  1.5-2.5  $\mu$ .

In the illustration (Fig. 2) the difference in form and habit of the

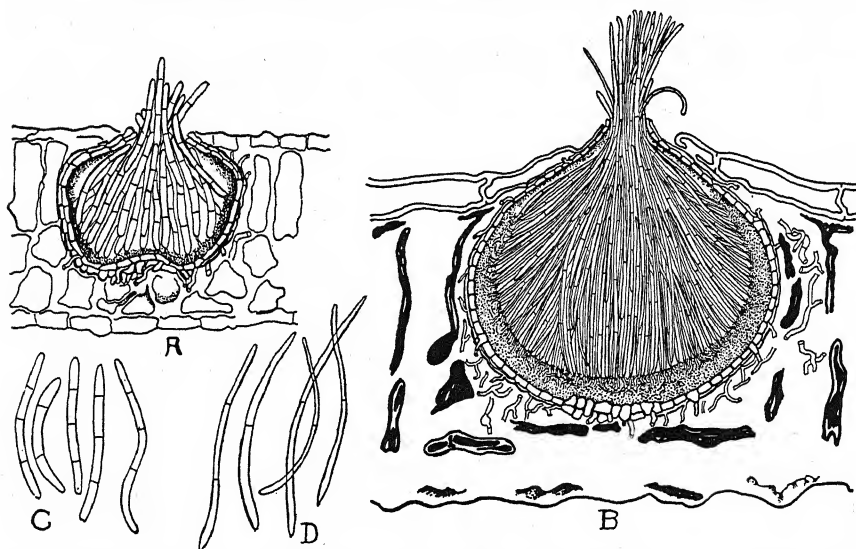


FIG. 2. A and B. Vertical sections of pycnidia of *Septoria brevispora* and *S. rubi*, respectively.  $\times 210$ . C and D. Pycnospores of *S. brevispora* and *S. rubi*, respectively.  $\times 325$ .

pycnidia is shown, while the extremes in spores are brought out much more in contrast than one often observes. The differences in spores in the 2 species are not great, although those of *Septoria brevispora* usually have greater diameter and are shorter than in *S. rubi*.

The differences in the pycnidia should be stressed. Those of *Septoria brevispora* approach what one might regard as a transition stage between

acervulus and pycnidium. Usually, the sporophores arise from the base, and often the sides of the pycnidium are perpendicular to the leaf surface giving a rather rectangular shape to the vertical section. Species having such pycnidia often are referred to *Phleospora*, but this one has been retained in *Septoria*, where it was originally placed by Saccardo. On the other hand, the pycnidia of *S. rubi* are flash-shape, always rounded, and with sporophores arising more than half-way up the sides. Figure 2, A, is a vertical section of a pycnidium of *S. brevispora* in a red-raspberry leaf (U. S. D. A. No. 9) and figure 2, B, is one of *S. rubi* in a leaf of Himalaya blackberry. The former was collected at Beltsville, Maryland, in October, and the latter at Corvallis, Oregon, in September. The pycnidia of both gave every evidence of maturity. Although the thickness of leaves of the 2 hosts may account for some of the difference in size of pycnidia of the 2 fungi, their morphology in other hosts is similar to that illustrated.

The susceptibility of *Rubus* in Oregon, where *Septoria rubi* is prevalent, and in Maryland where *S. brevispora* predominates is illustrated in parallel columns in table 1. In some varieties the difference in susceptibility is not marked; but in many cases, such as Chief, Cumberland, Farmer, Latham, Lloyd George, Newburgh, and Potomac, the great differences in this respect are more than could be explained on the basis of climatic or other ecological factors, in view of the fact that conditions in Oregon are favorable for severe

TABLE 1.—Relative susceptibility of certain varieties and species of *Rubus* to *Septoria* leaf spots in Oregon and Maryland

Variety or species U. S. D. A. No. 9 (Latham × Ranere)	Degree of spotting in Oregon ( <i>S. rubi</i> )	Degree of spotting in Maryland ( <i>S. brevispora</i> )
Lloyd George .....	Usually too slight to be noticeable	Severe
Latham .....	" " " " " "	Extreme
Potomac .....	" " " " " "	Very severe
Cumberland .....	None	" "
Farmer .....	"	Extreme
Chief .....	"	"
Viking .....	Practically none	Very severe
Newburgh .....	" "	Extreme
Loganberry .....	None	"
Youngberry .....	Severe	"
Van Fleet .....	Mild	Severe
June .....	None	"
Himalaya .....	"	Extreme
U. S. D. A. No. 458 (Viking × Lloyd George) .....	Severe Mild	..... .....

infection on certain varieties. The same varieties that are extremely susceptible in Maryland are quite resistant in Oregon. Again, if we are dealing with but one species of *Septoria*, why, for instance, should the variety

Lloyd George be resistant in Oregon and England and highly susceptible in Maryland and South Carolina? The difference doubtless is in the fungus, not in the climate.

Perhaps the final solution of the relationship between these 2 *Septorias* must lie in a complete knowledge of their perfect stages. The investigator has had no opportunity to study the ascogenous stage of *S. brevispora*, but was privileged to examine some of Roark's material upon which he based *Mycosphaerella rubi* Roark. The only location where Roark found *M. rubi* was Door County, Wisconsin, a peninsula between Green Bay and Lake Michigan proper.

Some question arises concerning the perfect stage of *Septoria rubi* West. Saccardo<sup>4</sup> suggested it to be *Sphaerella ligea* Sacc. Wolf,<sup>5</sup> who is familiar with *Septoria rubi* and *Mycosphaerella rubi* Roark, both of which he has collected, says "*S. ligea* is not the perfect stage of *Septoria rubi* Westd." It "develops in irregular brown lesions on green leaves."

In Oregon, *Sphaerella ligea* occurs either in whitish spots on green overwintering leaves or on fallen leaves, in March. It answers perfectly the description given by Saccardo, and has been found in plantings of Himalaya, Logan, and Evergreen (*Rubus laciniatus*) blackberries, where the European type of *Septoria rubi* occurs. Although their organic connection seems apparent by association, the writer has never successfully proved it by cultural method. We have never found *S. brevispora* in Oregon, but a collection of it on blackberry was taken as far west as Madison, Wisconsin, July 30, 1931, by C. E. Owens. We have never seen, in Oregon, any *Mycosphaerella* on *Rubus* similar to *M. rubi* Roark. The spores of the Wisconsin material (Roark) are very narrow ( $3.5-4.25\ \mu$ ), while in ours (*Mycosphaerella ligea*) they are  $5-7\ \mu$  broad and  $17-22\ \mu$  long.

#### SUMMARY

The extreme difference in susceptibility of certain *Rubus* species and varieties to *Septoria* leaf spot in Oregon and that found commonly in Maryland and North Carolina has brought to attention the occurrence of 2 species of *Septoria* on *Rubus* in America. These are *S. rubi* West. and *S. brevispora* (Sacc.) Zeller. *S. rubi*, as it occurs in America, seems identical to that in Europe. It occurs typically in Pacific Coast States and eastward, some having been definitely identified as far to the southeast as North Carolina. The geographic distribution of *S. brevispora* needs further study, but collections have been taken from Wisconsin, New York (*type*), Maryland, and North Carolina. It is *extremely* active in these southeastern States. Varieties of *R. occidentalis*, *R. strigosus*, and *R. idaeus* are for the most part extremely susceptible to *S. brevispora* and resistant to *S. rubi*, while blackberry species are rather generally attacked by both.

<sup>4</sup> Saccardo, P. A. Sylloge Fungorum. v. 1. Padua, 1882. (see p. 483.)

<sup>5</sup> Wolf, F. A. The perfect stage of *Cercospora rubi*. Mycologia 27: 347-356. 1935.



Diagnostic characteristics based on the leaf spots produced and on the imperfect stages are included for both species. There is some question as to the perfect stage of *S. rubi*, and that of *S. brevispora* has not been observed by the writer.

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## CERCOSPORA ORYZAE ON RICE IN THE UNITED STATES

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(Accepted for publication July 30, 1937)

A glume spot of rice in Japan caused by *Cercospora oryzae* Miyake was described and the causal fungus named by Miyake (1) in 1910. Subsequent reports on the disease in Japan have been made by Sawada (2, 3) and others. According to Wood (5) the disease also occurs in China, East Indies, Burma, Dominican Republic, Brazil, Puerto Rico, Cuba, and Louisiana. The report on the disease in Louisiana was made by Edgerton in 1920<sup>2</sup> but he did not determine the species of *Cercospora* causing the disease.<sup>3</sup>

A culture of *Cercospora* that proved to be morphologically identical with *C. oryzae* was isolated in February, 1934, from leaf spots found on second growth rice at the Rice Experiment Station, Crowley, La. Similar leaf spots were observed on rice in Alabama, Louisiana, Texas, and Arkansas during the 3 subsequent seasons and *C. oryzae* was cultured from the lesions.

Injury from this disease appears to be confined almost entirely to the reduction of the effective leaf area of the plant, although the fungus is found also on the sheaths, peduncle, and glumes. Evidence is lacking to indicate that the fungus causes a seedling blight or attacks the kernels. At present the disease is much more widespread in Arkansas, Louisiana, and Texas than is either the *Piricularia* or *Helminthosporium* leaf spot. The latter diseases, however, may cause greater damage than *Cercospora* in severely affected fields, because of the crown and culm injury caused by *Piricularia* and the flower and kernel injury caused by *Helminthosporium*.

### SYMPTOMS

Subsequent investigations have shown that the leaf, sheath, culm, and peduncle may be attacked, although Miyake (1) reported that the lesions were produced only on the glumes.

<sup>1</sup> Pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, in cooperation with the Arkansas, Louisiana, and Texas Agricultural Experiment Stations.

<sup>2</sup> Edgerton, C. W. Rice. U. S. Dept. Agr. Bur. Plant Indus. Plant Disease Bull. 4: 122. 1920. [Mimeographed.]

<sup>3</sup> Since the preparation of this paper a short report of the occurrence of *Cercospora oryzae* in Louisiana, by T. C. Ryker, has appeared in the Biennial Report of the Rice Branch Experiment Station, Crowley, La., for 1935-1936.

The symptoms previously reported by other investigators and verified by the writer are briefly as follows:

Leaf lesions are linear, 3 to 5 mm. in length, usually not exceeding 1 to  $1\frac{1}{2}$  mm. in breadth, the long axis with the long axis of the leaf. The center of the spot to the extent of  $\frac{1}{4}$  mm. is dark brown, the border fading as the outer margin of the spot is approached. The sheath lesions usually are the same as those on the leaf or may be a trifle larger, while those on the peduncle are narrower, and those of the glumes are usually shorter but more inclined to spread laterally (Fig. 1).

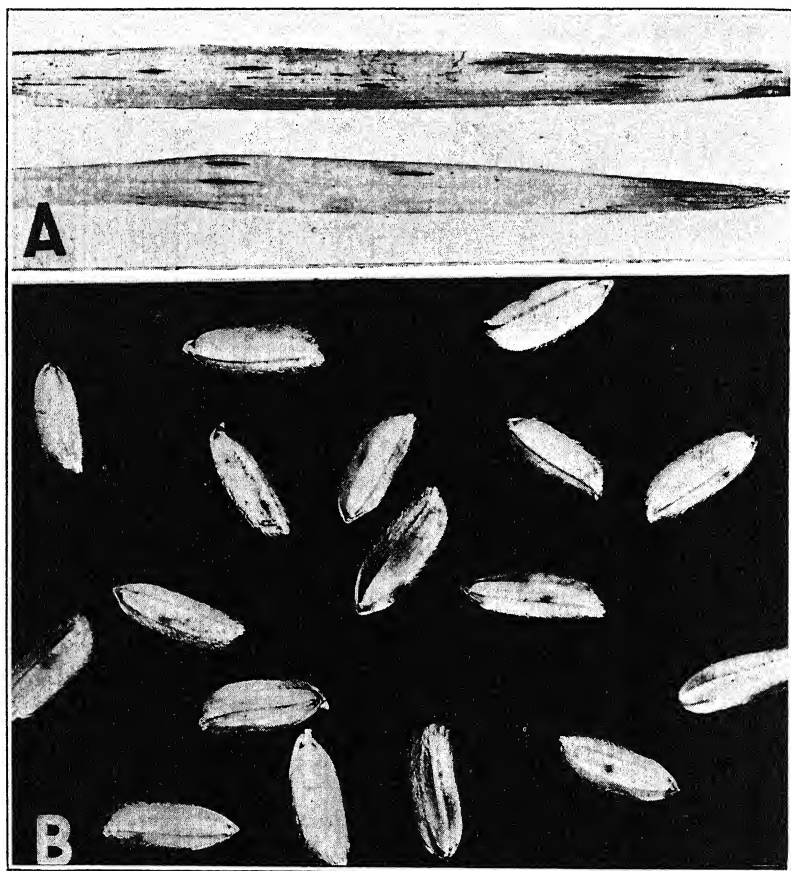


FIG. 1. A. Leaves of Blue Rose rice with lesions produced by *Cercospora oryzae*, from near Crowley, La.  $\times 1$ . B. Seed of Early Prolific rice from vicinity of Anahuac, Tex., with lesions produced by *Cercospora oryzae*.  $\times 2$ .

Variations in invasion due to variety produce slight differences in the macroscopic appearance of the lesions. In resistant varieties the lesions are

uniformly dark red brown throughout and very narrow, while in susceptible varieties the spots are wider with a narrow light brown or grey-brown center.

The elongate, lighter brown lesions produced by *Cercospora oryzae* are distinctly different from those produced by *Helminthosporium oryzae* Breda de Haan and *Piricularia grisea* (Cooke) Sacc. The latter fungi produce round, oval, or elongate spots, depending on the varietal response of the host, with a light brown to ashen center, surrounded by a dark red-brown border zone and concentric zones in the more susceptible varieties.

#### VARIETAL SUSCEPTIBILITY

The varieties of rice studied exhibit a much wider range of resistance to *Cercospora oryzae* than to *Helminthosporium oryzae*.

In 1935, at Texas Substation No. 4 (Beaumont), 61 varieties and selections and at the Rice Branch Experiment Station, Stuttgart, Ark., 34 of 109 varieties and selections appeared to be resistant. In 1936, the 61 varieties from Beaumont were grown under conditions ideal for infection and 39 of them still appeared to be resistant, while 19 of the 36 apparently resistant varieties at Stuttgart, Ark., continued to be resistant. The varieties that were resistant in both 1935 and 1936 include Nira, Tokalon, and C.I. Nos. 461, 2711, 2738, 4603, and 4966. The most susceptible of the commercial rice varieties are Blue Rose, Edith, Lady Wright, and Early Prolific.

#### INOCULATION EXPERIMENTS

The first inoculations were made on August 31, 1935. Twelve leaves of Blue Rose rice detached from plants growing in the open at Fayetteville, Ark., were placed in jars in water, in the inoculation chamber, and sprayed with water for 2 hours, after which they were inoculated with a water suspension of the conidia from a pure culture. The inoculated leaves, and controls similarly treated but not inoculated, were kept in the inoculation chamber for 24 hours and then moved to a greenhouse bench. Lesions typical of those produced under field conditions were evident on 6 of the inoculated leaves by September 14. Tissue platings were made from these lesions and a pure culture of the fungus was secured. Additional inoculations were made in February, March, and April, 1936, on Blue Rose, Early Prolific, Nira, Rexoro, Gin Bozu, C.I. Nos. 4700 and 5343, and selections from the crosses Fortuna  $\times$  Early Prolific and Edith  $\times$  Blue Rose. A few typical lesions appeared on these plants and the fungus was isolated by tissue platings. Infections were relatively infrequent under the conditions of these experiments.

#### PATHOLOGICAL HISTOLOGY

Typical leaf spots on Blue Rose rice growing under field conditions and naturally infected were killed and fixed in the same manner as previously

reported (4) for *Helminthosporium* leaf spot. Subsequent treatment was the same as for the *Helminthosporium* leaf-spot material. The specimens were sectioned, both cross and longitudinal sections being cut at 7 and 10  $\mu$ . Various stains were used but the best results were obtained with Haidenhain's iron alum haematoxylin and safranin-light green.

Infection apparently occurs through the stomata and the fungus becomes established in the parenchyma immediately beneath the stomata. Longitudinal spread occurs mainly in the epidermal cells. The conidiophores are produced from sub-stomatal branches of the hyphae. The mycelium is mostly intracellular, as very little was found in the intercellular spaces except at the points that conidiophores had been formed.

#### CONTROL

The fact that about 60 rice varieties and selections are apparently highly resistant to or immune from the fungus, suggests the possibility of developing resistant varieties by hybridization, that possess other desirable agronomic characters that may be used to supplant present susceptible varieties.

#### SUMMARY

*Cercospora oryzae* has been found in recent years in great abundance on the sheaths, leaves, peduncles, and glumes of rice in Arkansas, Alabama, Louisiana, and Texas.

The lesions produced by *Cercospora oryzae* are narrower and lighter brown than those produced by *Helminthosporium oryzae* or *Piricularia oryzae*.

Fifty-eight varieties and hybrid selections were resistant in 2-year tests.

The pathogenicity of the fungus was established by inoculation and subsequent isolation of the fungus from typical lesions produced on several varieties of rice.

The fungus appears to be localized in the epidermal region of the host and conidia are produced on conidiophores that grow through the stomata.

Control may be effected by developing resistant varieties.

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# VARIETAL REACTION OF PEA TO PEA-STREAK VIRUS 1

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## INTRODUCTION

In the fall and winter of 1934 a new virus disease of peas, *Pisum sativum* L., was observed in the greenhouse at Arlington Experiment Farm, Rosslyn, Virginia. It was first noted about 3 weeks after a release of the pea aphid, *Illinoia pisi* Kalt., on greenhouse-grown peas, and, after 5 more weeks, it was widely disseminated throughout the planting. The aphids that were released were collected from what appeared to be healthy field-grown alfalfa, *Medicago sativum* L. Examination of the alfalfa the following spring showed some of the plants infected with an alfalfa-mosaic virus. It was first reported that this pea disease was caused by an alfalfa mosaic virus, but it has since been proved that the virus is distinct from that of alfalfa mosaic. Without adequate proof of the exact source of the virus, it was assumed that the collected pea aphids carried and transmitted it to the peas growing in the greenhouse. The specificity of the disease was first suspected from the fact that the symptoms differed from other known viroses affecting pea. The disease caused by the virus was described by Zaumeyer and Wade<sup>1</sup> and later identified by Zaumeyer<sup>2, 3</sup>, who compared it with several strains of the alfalfa-mosaic virus. Because of the characteristic symptoms produced on pea, it was named pea-streak virus 1. It was found to be unlike other viruses affecting pea. The purpose of this paper is to record the reaction of a number of pea varieties to this virus.

## METHODS

The studies were conducted in the greenhouse at a temperature of approximately 60° to 70° F. Forty-seven pea varieties were subjected to the virus, 32 of which were tested both in 1934 and 1936. Fifteen others were grown for only one year, either in 1934 or 1936 (Table 1). Twenty-five seeds of each variety were planted in greenhouse benches in each of 4 replicated plots. The Broad Windsor bean was planted at intervals of 5 rows throughout the planting. In 1934 field-collected pea aphids, reared in sufficient numbers in screened cages, were placed on the Broad Windsor beans and later migrated, or were artificially transferred to the peas. The disease,

<sup>1</sup> Zaumeyer, W. J., and B. L. Wade. A pea streak caused by alfalfa mosaic. (Abstract) *Phytopath.* 26: 114. 1936.

<sup>2</sup> Zaumeyer, W. J. Pea streak and its relationship to strains of alfalfa mosaic. (Abstract) *Phytopath.* 27: 144. 1937.

<sup>3</sup> Zaumeyer, W. J. A streak disease of peas and its relationship to several strains of the alfalfa mosaic virus. *Jour. Agr. Res.* [U.S.]. (In press.)

TABLE 1.—*The reaction of varieties of peas to pea streak virus 1*

Number	Variety	Survival values		
		1934	1936	Average
1	Nott Excelsior .....	3.750	3.155	3.453
2	Little Marvel .....	3.473	2.598	3.035
3	Laxton Progress .....	3.555	2.373	2.964
4	Profusion .....	3.553	2.183	2.868
5	Hundredfold .....	3.570	2.113	2.841
6	Bruce .....	3.500	2.168	2.835
7	Surprise .....	3.075	2.575	2.825
8	Long Pod Alaska .....	3.318	1.990	2.654
9	Dwarf Telephone .....	3.153	2.120	2.636
10	Lincoln .....	2.790	2.315	2.553
11	White Eye Marrowfat .....	2.765	2.135	2.450
12	Short Admiral .....	2.378	2.408	2.393
13	Austrian Winter .....	2.330	2.450	2.390
14	Premium Gem .....	2.658	2.043	2.350
15	World Record .....	2.905	1.710	2.308
16	Pride of the Market .....	3.225	1.283	2.254
17	Green Admiral .....	2.235	2.255	2.245
18	Onward .....	3.268	1.218	2.243
19	Alaska .....	3.018	1.430	2.224
20	Giant Stride .....	2.540	1.853	2.196
21	Black Eye Marrowfat .....	3.003	1.353	2.178
22	Alderman .....	2.654	1.703	2.174
23	Horsford .....	2.855	1.350	2.103
24	Telephone .....	2.345	1.843	2.094
25	Thomas Laxton .....	2.860	1.280	2.070
26	Green Giant .....	1.583	2.248	1.915
27	Capucijner .....	2.015	1.763	1.889
28	Potlatch .....	2.048	1.713	1.880
29	Perfection .....	2.228	1.410	1.819
30	Stratagem .....	1.885	1.710	1.798
31	Champion of England .....	2.423	1.023	1.723
32	Phenomenon .....	2.078	1.180	1.629
33	Senator .....	3.665	.....	.....
34	Gradus .....	2.603	.....	.....
35	New Late Gradus .....	2.173	.....	.....
36	Dwarf Quite Content .....	1.768	.....	.....
37	Horal .....	.....	2.833	.....
38	Yellow Admiral .....	.....	2.563	.....
39	Creole .....	.....	2.233	.....
40	White Canada .....	.....	2.023	.....
41	Bluebell .....	.....	1.758	.....
42	Maryland Alaska .....	.....	1.725	.....
43	Harrison Glory .....	.....	1.703	.....
44	Prince of Wales .....	.....	1.615	.....
45	Swedish Yellow .....	.....	1.515	.....
46	Maple .....	.....	1.390	.....
47	Mammoth Melting Sugar .....	.....	0.948	.....

as mentioned previously, appeared a few weeks afterward and spread throughout the planting.

In 1936 both Broad Windsor beans and peas were mechanically inoculated with the virus and the pea aphid was again released in the greenhouse to insure complete virus dissemination. The greenhouse was fumigated at

about 3-week intervals to destroy the aphids. They were again introduced about a week following the fumigation. At the end of 8 weeks, the data were recorded.

The inoculum for the mechanical insulation was prepared by crushing infected plants in a sterile mortar and straining the juice through cheesecloth. Young plants were dusted with carborundum powder and then inoculated by rubbing the leaves with a pad immersed in the inoculum.

#### SYMPTOMS OF THE DISEASE

The symptoms of this disease have been described previously<sup>3</sup> and are redescribed here only briefly. The initial symptom, which appears about 13 to 15 days following inoculation, is a slight purpling and streaking of the stem, more particularly at the base of the stipule. This is followed by a recurving of the stipules, a streaking of the petioles, and a downward curling of the leaflets. The veins first show a clearing, but are later discolored and the plant becomes slightly chlorotic. The internodes above the point of inoculation are shortened and the growing tip is frequently rosetted; later, this portion of the plant may die, followed by the death of the entire plant in many instances. All infected plants are not killed, but those that survive are decidedly dwarfed and malformed. The stem of a seriously infected plant may be streaked and the discoloration may extend from the base to the tip of the plant. Internal necrosis is very common. The pods that are formed before the plant becomes seriously infected are spotted and pitted and much smaller than normal. Such infected pods seldom reach maturity.

#### EXPERIMENTAL RESULTS

The varieties of peas that were tested (Table 1) were grown in 4 series in the greenhouse in 1934 and 1936. These were randomized within the series

TABLE 2.—*Analysis of variance of reaction of pea varieties to pea-streak virus 1, for the years 1934 and 1936*

Variation due to	Degrees of freedom	Sum of squares	Mean square	Standard deviation
Blocks .....	3	2.24	0.7467	.....
Varieties .....	31	45.78	1.4768	.....
Years .....	1	49.28	49.2800	.....
Years $\times$ varieties	31	25.72	0.8297	.....
Blocks $\times$ years .....	3	9.14	3.0467	.....
Error .....	186	87.94	0.4728	0.688
Total .....	255	220.10	0.8631	.....

<sup>a</sup>  $F_v = 3.12$  when referred to error. 1 per cent point = 1.23.

$SE_{M_4} = 0.344$ . Significant difference = 0.973.

$SE_{M_8} = 0.243$ . Significant difference = 0.687.

and the statistical constants computed in a variance-analysis arrangement (Table 2). The standard error of the mean for the reaction of 8 plots was found to be 0.243 (Table 2). For a difference to be considered significant, it should exceed 0.687 (Table 2). As mentioned previously, in addition to the 32 varieties tested in each of the 2 years, 4 other varieties were included in 1934 and 11 others in 1936 (Table 1). Variance analysis for the 36 and 43 varieties, respectively, are shown in tables 3 and 4. The standard error of the mean of 4 plots is 0.351 for 1934, and 0.378 for 1936, with minimum significant differences of 0.992 and 1.068, respectively (Table 3).

TABLE 3.—*Analysis of variance of reaction of pea varieties to pea-streak virus 1, 1934*

Variation due to	Degrees of freedom	Sum of squares	Mean square	Standard deviation
Blocks .....	3	4.72	1.5733	.....
Varieties .....	35	49.04	<sup>a</sup> 1.4011	.....
Error .....	105	51.71	0.4925	0.7018
Total .....	143	105.47	0.7376	.....

<sup>a</sup>  $F_v = 2.84$  when referred to error. 1 per cent point = 1.38.

$SE_{M_4} = 0.351$ . Significant difference 0.992.

In table 1, the 32 varieties are arranged in order of their indicated resistance to pea-streak virus 1, based on the average of 8 plots in 2 years. The 14 varieties grown only 1 year are also listed in table 1, but they are not included in the ranking with the varieties grown for the 2 years. A plant showing no symptoms at the end of 8 weeks was recorded as healthy and given a rating of 4, mildly infected plants were rated 3, more severely infected 2, very severely 1, and dead plants 0. The number of plants in a category multiplied by the survival assigned to that class and the sums of these values for all classes occurring in a plot were recorded as total survival value. Since the number of plants varied from plot to plot, depending upon differences in germination, occasional death of seedlings due to root rot, *Fusarium martii* var. *pisi*, and *Pythium* sp., the total survival value of each plot was divided by the number of plants in the plot and this average recorded as the survival value of the plot. Table 1 shows the values for the average of 4 plots in 1934 and in 1936 and the 2-year averages of the varieties tested both years.

Nott Excelsior (Table 1) was the most resistant variety, followed closely by Little Marvel. Since minor symptoms are somewhat difficult to distinguish there may be no actual difference in resistance of these two varieties. The difference between the mean reactions is not sufficiently large to indicate a statistically significant difference. The most susceptible variety in 1934 was Phenomenon, with a survival value of 1.629. In the 1936 experiment, Mammoth Melting Sugar was the most susceptible of any of the varieties



tested, having a survival value of only 0.948. There is no correlation between earliness and resistance; the first 3 high ranking varieties are early but the fourth is at least 2 weeks later, while the very early variety Alaska ranks nineteenth. There is no statistical significance between any two of the survival averages of the first 7 varieties listed, but there is a significant difference between the first and eighth and between the first and any of the varieties following the eighth.

TABLE 4.—*Analysis of variance of reaction of pea varieties to pea-streak virus 1, 1936*

Variation due to	Degrees of freedom	Sum of squares	Mean square	Standard deviation
Blocks .....	3	13.70	4.5667	.....
Varieties .....	42	42.88	<sup>a</sup> 1.0210	.....
Error .....	126	71.82	0.5700	0.7550
Total .....	171	128.40	0.7509	.....

<sup>a</sup>  $F_v = 1.79$  when referred to error. 1 per cent point = 1.32.

$SE_{M_4} = 0.378$ . Significant difference 1.068.

As mentioned earlier, the plants in the 1934 experiment were not artificially inoculated. The disease was transmitted and disseminated entirely by the pea aphid. As a result, the infection was not so widespread as it was in 1936 when the seedlings were mechanically inoculated, followed by natural aphid spread. This difference in method probably accounts for the much higher level of survival in 1934 than in 1936 (Table 1). The mean survival value for 128 plots in 1934 is 2.782, but in 1936 this was reduced to 1.904, which is approximately 68 per cent of the 1934 value for the same varieties. This difference amounts to  $0.778 \pm 0.090$ , which is highly significant. It is of interest to note, however, that those varieties that rated high in 1934 usually rated fairly high in 1936. Nott Excelsior ranked first in both years.

#### SUMMARY

Experiments were conducted under greenhouse conditions in 1934 and 1936, to determine the varietal susceptibility of 47 varieties of peas to pea streak virus 1. The statistical constants were computed in a variance analysis. All varieties tested were susceptible to the virus disease. In the 2 years' trial, Nott Excelsior and Little Marvel varieties showed the most resistance, while Champion of England and Phenomenon were the most susceptible. In the 1936 trial alone, Mammoth Melting Sugar was the most susceptible of any of the varieties tested.

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# THE INACTIVATION OF THE ORDINARY TOBACCO-MOSAIC VIRUS BY MICROORGANISMS

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(Accepted for publication August 18, 1937)

The unusual resistance of the tobacco mosaic virus (*tobacco virus 1*) (2) to natural destruction outside of the living host, challenges investigation of the factors concerned with its eventual inactivation. Although the nature of the comparatively rapid loss of infectivity of numerous other plant viruses under similar circumstances is not fully understood, it is fairly obvious that in most instances the factors differ from those concerned in the inactivation of *tobacco virus 1*. The "short-lived" viruses are apparently incapable of retaining infectivity for more than a relatively few hours or days outside the living host cell, regardless of other circumstances, whereas *tobacco virus 1* may be maintained in an active condition for many years under a variety of storage conditions.

A number of different agencies, however, may inactivate the tobacco mosaic virus. The action of heat, toxic chemicals, light, and adsorption have long been known. Less specific factors, such as desiccation and freezing of free virus in the soil, and "weathering" in tissue, have been shown to cause inactivation in nature (1, 4). The destruction of the virus in decomposition of plant tissue is clearly an important natural agency of much scientific and practical interest. The present investigation is devoted largely to a preliminary survey of inactivation by bacteria and fungi under pure-culture conditions. Aside from some limited trials by Mulvania (6) in this laboratory about 12 years ago, little, apparently, has been done on this subject. The "local lesion" method of determining the virus concentration was not available at the time of Mulvania's work, and the number of plants used in his tests was not sufficiently large to yield good comparative data. Nevertheless, inactivation of the virus by pure cultures of 3 or 4 bacterial species was fairly obvious from the data presented.

The present results show that a wide variety of microorganisms in pure culture are capable of inactivating *tobacco virus 1*. The efficiency or rate of inactivation may, however, vary greatly with the species concerned.

## EXPERIMENTAL METHODS

Under natural conditions, the destruction of the virus occurs largely in the dead plant tissues, but may not take place until after the virus is extracted or leached from the tissues. It was hence thought worth while to investigate inactivation in both plant tissue and in plant extract.

The virus was prepared for the tests in both sterile leaf tissue and in sterile plant extract. The latter was prepared in the usual manner by filtra-

<sup>1</sup> Deceased Dec. 28, 1936.

tion of extract from mosaic tobacco plants through bacteria-proof filters. One cc. portions of these extracts usually were added to 5 cc. of sterile nutrient broth in tubes under aseptic conditions, although, in some instances, the pure plant extracts also were used as culture media.

The sterile plant tissues were prepared by cutting  $\frac{3}{8}$ -inch disks from distinctly mosaic-affected large green tobacco leaves. These disks, 50 to 60 at a time, were rinsed in 5 to 7 changes of sterile water in 500 cc. Erlenmeyer flasks. The disks were then placed singly on dry filter paper in sterile Petri dishes and dried for 24 hours at about 40° C. They were then placed in a dry-air oven, usually set at 85° C., and left for 3 hours' disinfection by heat. After many trials, this temperature and time of exposure were found to give an average of about 300 virus lesions per disk; and, when dropped individually into tubes of nutrient broth, to yield about 50 per cent of the disks free from contaminating organisms. A much higher percentage of sterile disks may be secured by a preliminary disinfection of the leaves with mercuric chloride (1-1000) for 1-2 minutes, followed by washing before cutting the disks. This treatment, although used in only 2 instances, seemed not to affect the subsequent results.

When the disks were found sterile, they were either inoculated while in the broth, or transferred to agar slants before inoculation. Ordinarily, nutrient broth was used for the bacteria, and potato agar for the fungi. The inoculations with the pure cultures usually were made in triplicate.

The species of bacteria and fungi used were secured from reliable sources; that is, usually from persons actively working with the organisms. The identity of some of the cultures was verified during the course of the experiments, but others were not verified beyond cultural characteristics. The writers are indebted to members of the Department of Plant Pathology and Agricultural Bacteriology of the University of Wisconsin for most of the cultures. The following organisms were used:

*Bacteria*: *Aerobacter aerogenes* (Kruse) Bergey et al., *Bacillus mycoides* Flügge, *B. radiobacter* Beij. and Van Deld, *B. subtilis* (Ehrenberg) Cohn, *Cytophaga hutchinsoni* (Winogradsky) Bergey et al., *Erwinia carotovora* (Jones) Holland, *Escherichia coli* (Migula) C. and C., *Phytomonas angulata* (Fromme and Murray) Bergey et al., *P. insidiosa* (L. McC.) Bergey et al., *P. phaseoli* (Erw. Smith) Bergey et al., *P. tabaci* (Wolf and Foster) Bergey et al., *P. tumefaciens* (Smith and Townsend) Bergey et al., *P. stewartii* (Erw. Smith) Bergey et al., *Proteus vulgaris* Hauser, *Pseudomonas fluorescens* (Flügge) Migula, *Sarcinia flava* De Bary, *S. lutea* Schröter, *Serratia keilensis* (L. and N.) Bergey et al., *S. marcescens* (Bizio) Bergey et al., *Staphylococcus aureus* Rosenbach.

*Fungi*: *Actinomyces scabies* (Thax.) Güssow, *Aspergillus flavus* Link, *A. niger* Van Tiegham, *A. ochraceus* Wilhelm, *A. terreus* Thom, *Botrytis*

*cinerea* Pers., *Cercospora nicotianae* Ell. and Ev., *Chaetomium globosum* Kunze, *Diplodia zeae* (Schw.) Lév., *Fusarium lycopersici* Brushi, *F. orthoceras* App. and Wr. var. *pisi* Linford, *F. oxysporum* Schl., *Gibberella saubinetii* (Mont.) Sacc., *Glomerella cingulata* (Stoneman) Sp. and Von S., *Helminthosporium gramineum* Rab., *Macrosporium solani* Ell. and Mart., *Penicillium baiiolum* Biourge, *Pilobolus* sp., *Pyronena* sp., *Rhizoctonia* sp., *Sclerotinia fructigena* Pers. (Schroet.), *Septoria lycopersici* Speg., *Thielaviopsis basicola* (B. and Br.) Ferr., *Trichoderma* sp., *Venturia inaequalis* (Cooke) Winter.

*Yeast: Sacchromyces* sp. (var. Red Star).

The cultures containing the virus were all incubated at a room temperature of 21–25° C. Samples of each lot of the sterile disks and extracts usually were tested before inoculation with the organisms to determine the virus concentration present, and other lots were kept as noninoculated controls to be tested at regular intervals, along with the inoculated series of tubes. The disks were removed from the tubes (with fungus mat, if necessary) and thoroughly ground in a mortar with 3 drops of water. This was diluted with 5 cc. of water and wiped over 6 leaves of the hybrid (*Nicotiana tabacum* × *N. glutinosa*) host plant (3). The 1 cc. extract cultures in broth were made up to 10 cc. with water before inoculation to the host plant.

After 3 or 4 days (at about 85° F.) the local lesions on the 3 leaves of the host showing the maximum number of spots were counted, as representing the relative concentration of the virus in the disk or extract tested. When such tests are conducted on suitably grown greenhouse plants selected for comparative size, the results are fairly comparable and reliable, especially if made on duplicate plants. The chief variable factor appears to be between plants grown at different periods of the year, which difficulty cannot be easily overcome by either replications or statistical methods.

Some modifications in methods of study were used, the chief of which consisted in providing aeration to the cultures by means of a 2-arm culture tube on a mechanical rocker (Fig. 1). In contrast to this, the rate of inactivation of the virus was also compared in virus-infested soil and sand under anaerobic conditions.

#### EXPERIMENTAL RESULTS

Approximately 2500 inoculations to the hybrid host were made in this study, but only a portion of the data can be presented profitably at this time. The results shown in the tables are to be interpreted in relative terms, as they do not necessarily represent the actual number of virus particles present in any sample. Five-eighths-inch green disks from mosaic leaves, ground up and diluted with 1000 cc. of water, in 12 determinations, yielded an average of 258 lesions (range 207–392) on 3 leaves of the hybrid host. Estim-

ing 2 cc. of inoculum as sufficient to inoculate 3 leaves, 1 disk would contain at least enough virus particles to produce 129,000 lesions. In our experiments, over 99 per cent of this virus was usually inactivated in the disks by the heat treatments to which they were subjected before inoculations were made. This rather severe treatment was desirable in part for the purpose of reducing the virus present to a practical concentration for making counts without extensive dilution. A part of this inactivation was probably not thermal in nature, but may have been the result of fixation or clumping of the virus particles as a result of drying. A preliminary drying at 45° C.,

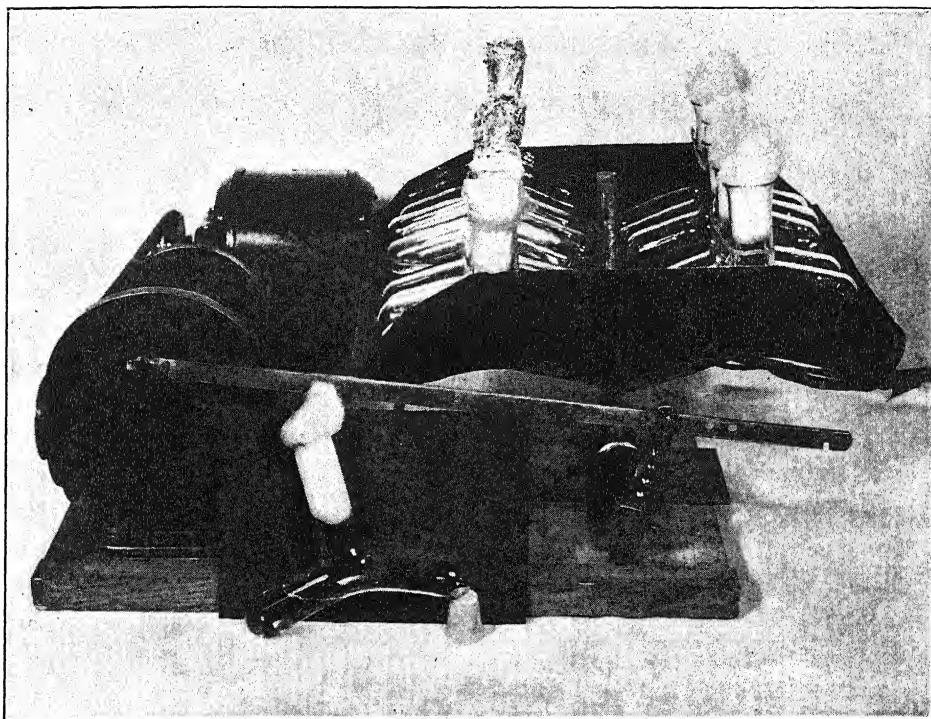


FIG. 1. The two-arm culture tube (in foreground) used for aerating sterile virus extract and cultures of microorganisms on virus extracts. The continuous mechanical rocker consists of motor, speed-reducing gear, rocker arm, and rack for holding 16 two-arm culture tubes.

for example, reduced the number of lesions from an average of 258 to 101, at a 1-1000 dilution. Further heating to 85°-86° C., for 3 hours reduced the number to an average of about 300 lesions on 3 leaves at a 1-5 dilution (average of 38 trials). Since some disks used in the experiments were heated to different temperatures for different lengths of time, considerable variation from the average occurs and the disks are actually comparable only with their respective controls.

A large number of the disks in the culture tubes naturally were contaminated with apparently pure cultures of a great variety of bacteria and fungi of undetermined species. These tubes were retained and used for preliminary inoculation trials on the host at various intervals. The chief purpose of these tests was to give a suggestion as to the approximate time that might be required by the known species to inactivate the virus in the cultures. The tests showed at the outset that the bacteria, as a group, might not inactivate appreciable amounts of the virus in the disks over a period of 3 or 4 months, but that the fungi were distinctly more efficient inactivators (Table 1), though they frequently did not completely inactivate all of the

TABLE 1.—*Summarized results of inoculations, comparing the inactivating action of various undetermined species (contaminants) of bacteria and fungi on tobacco virus 1 in leaf disks in culture tubes*

Culture tubes	Number of cultures tested	Average number of lesions on 3 hybrid leaves
With bacteria .....	94	251
With fungi .....	101	58
Sterile controls .....	112	338

virus after several months. Some of the experiments with the known species in pure cultures were, therefore, carried for as long as 120 days before the final inoculations were made. The effect of certain representative bacteria

TABLE 2.—*A comparison of the inactivating action of various bacteria (in broth) and of certain fungi (on agar) on tobacco virus 1 in leaf disks*

Organism	Number of lesions on 3 hybrid leaves from			
	Inoculated disks after		Sterile control disks after	
	90 days <sup>a</sup>	120 days <sup>a</sup>	1 to 5 days <sup>b</sup>	90 to 120 days <sup>a</sup>
<i>Bacillus radiobacter</i> .....	400	325	317	246
<i>Phytomonas tumefaciens</i> .....	340	400	317	246
<i>Staphylococcus aureus</i> .....	600	400	700	239
<i>Phytomonas phaseoli</i> .....	264	236	157	136
<i>Pseudomonas fluorescens</i> .....	270	144	252	158
<i>Phytomonas stewartii</i> .....	125	170	186	286
<i>Bacillus mycoides</i> .....	46	135	126	350
<i>Cytophaga hutchinsoni</i> .....	32	108	350	350
<i>Phytomonas angulata</i> .....	135	57	353	297
<i>Escherichia coli</i> .....	25	8	475	600
<i>Aspergillus ochraceus</i> .....	51	4	650	700
<i>Penicillium baiiolum</i> .....	29	3	278	363
<i>Trichoderma</i> sp. ....	30	2	330	326
<i>Aspergillus flavus</i> .....	6	5	135	297
<i>Botrytis cinerea</i> .....	1	1	323	104
<i>Septoria lycopersici</i> .....	0	0	135	156

<sup>a</sup> Single disks.

<sup>b</sup> Average of 2 to 4 disks.

and fungi in cultures containing *tobacco virus 1* in leaf disks is shown in table 2. Only one bacterial species in this group, namely *Escherichia coli*, showed marked inactivation of the virus, whereas the fungi, as a group, almost completely inactivated it. The condition of the disks largely explained the result. The fungi, being good cellulose decomposers, readily broke down the leaf tissue and, having better access to the virus particles themselves, probably continued to inactivate them. On the other hand, the bacteria as a group usually were unable to break down the cell structure, and consequently failed to reach directly the virus particles, which, as will be shown, are often quite susceptible to inactivation by bacterial organisms when free from plant tissue.

It also seems likely that the bacterial enzymes or toxins produced in the cultures either were not able to penetrate the cell tissue or, as is more likely, were not capable of inactivating the virus after penetration occurred. The results in table 2 are not conclusive as to the complete failure of any one bacterial species to inactivate the virus in leaf disks; but, with supporting data not shown here, it seems probable that such organisms as *Bacillus radiobacter*, *Phytomonas tumefaciens*, and *Staphylococcus aureus* are practically without effect under these circumstances. Whether or not *Escherichia coli* and *Phytomonas angulata* are active because of cellulose decomposing ability is not known, but this is no doubt the case with *Cytophaga hutchinsoni*. *Aerobacter aerogenes* was shown in subsequent tests to inactivate the virus in disks considerably more effectively than *E. coli*.

When the virus in the form of filter-sterile extract in broth is exposed to the action of pure cultures of bacteria, it is clear that some species are fairly rapid and good inactivators when in direct contact with the virus particles (Table 3). Considerably greater variation in behavior between

TABLE 3.—The inactivation of tobacco virus 1 in filter-sterile extract (in nutrient broth) by various bacteria

Organism	Number of lesions on 3 hybrid leaves from				
	Inoculated extracts after			Sterile control extracts after	
	19 days	23 days	67 days	1 day	23 to 67 days
<i>Phytomonas tabaci</i> .....		15	30	255	320
<i>Serratia marcescens</i> .....	5	9	0	"	345
<i>Escherichia coli</i> .....	6	64	19	"	360
<i>Proteus vulgaris</i> .....	6	176	5	"	360
<i>Aerobacter aerogenes</i> .....	0	0	0	800	900
<i>Bacillus subtilis</i> .....	51	8	36	"	400
<i>Sarcinia lutea</i> .....	500	78	250	"	900
<i>Bacillus radiobacter</i> .....	185	455	450	"	1068
<i>Erwinia carotovora</i> .....	500	6		"	900
<i>Phytomonas phaseoli</i> .....	300	340	338	700	545
<i>Cytophaga hutchinsoni</i> .....	750	49	190	"	350
<i>Bacillus mycoides</i> .....	800	110	212	"	400

the species may, however, be noted than appears to be the case with the fungi on the leaf disks. *Bacillus radiobacter* and *Phytophthora phaseoli* are distinctly slow in action, as is perhaps *Bacillus mycoides* and *Sarcinia lutea*. *Aerobacter aerogenes* is evidently the most rapid inactivator of the virus, although several other species may closely approach it. In contrast, table 4

TABLE 4.—*The inactivation of tobacco virus 1 in leaf disks by various fungi in pure culture in nutrient broth*

Fungus	Number of lesions on 3 hybrid leaves from				
	Inoculated disks after			Sterile control disks after	
	60 days	67 days	86 days	1 to 5 days <sup>b</sup>	60 to 86 days <sup>a</sup>
<i>Cercospora nicotianae</i> .....	18	4		132	60
<i>Pyrenopeziza</i> sp. ....	5	1	5	126	141
<i>Helminthosporium gramineum</i> .....	5	37		98	163
<i>Macrosporium solani</i> .....	12	0	8	98	266
<i>Fusarium oxysporum</i> .....	3	3	5		133
<i>Pilobolus</i> sp. ....	2	3	2	132	112
<i>Diplodia zeae</i> .....	78	0	0		133
<i>Penicillium bailloum</i> .....	1	22	5	175	190
<i>Aspergillus ochraceus</i> .....	5	0	3	185	216
<i>Botrytis cinerea</i> .....	13	7	6	185	138
<i>Trichoderma</i> sp. ....	15	9	81	175	204
<i>Chaetomium globosum</i> .....	0	3	0	260	204
<i>Aspergillus niger</i> .....	23	8	0	260	96
<i>Aspergillus terreus</i> .....	0	6	4	175	190
<i>Aspergillus flavus</i> .....	12	48	1	"	"
<i>Venturia inaequalis</i> .....	0	1	0	"	"
<i>Sclerotinia fructigena</i> .....	0	0	2	179	198
<i>Gibberella saubinetii</i> .....	0	3		"	"
<i>Fusarium orthoceras</i> .....	26	13		"	"

<sup>a</sup> Single disks.

<sup>b</sup> Average of 2 disks.

shows the action of a variety of fungi on leaf disks in nutrient broth and, except for some minor variations, which may be in part accounted for by the difficulty of maintaining the disks at the surface in liquid media, the inactivation is fairly uniform, at least after 60 days. When the rate of inactivation over shorter periods was determined, greater differences were found, although the results were not easily interpreted on account of the different rates at which the fungi began active growth following transfer. Taking all the data into consideration, it seemed probable that certain *Fusaria* were considerably less rapid inactivators than were such fungi as *Sclerotinia fructigena* or even *Venturia inaequalis*. On the other hand, such an effective cellulose-decomposer as *Trichoderma* was not unusually destructive to the virus.

It was not expected that the use of fungi on virus extract in broth culture would prove a good method for determining the inactivating action of fungi,



because of the common tendency of the fungi to grow profusely only on the surface of liquid media. Sufficient tests, consequently, were not set up, but limited data indicate that certain fungi, at least, may completely inactivate the virus in liquid culture.

It is, of course, to be recognized that the results secured may vary considerably with the incubation temperatures. For example, *Aerobacter aerogenes* evidently has a much wider temperature range than *Bacillus subtilis* (Table 5). It also is to be recognized that inactivation, in nature, is

TABLE 5.—*The influence of temperature on the rate of inactivation of tobacco virus 1 by certain bacteria and fungi*

Organism	Virus in	Number of lesions on 3 hybrid leaves after incubation					
		14 days			28 days		
		13° C.	20° C.	28° C.	13° C.	20° C.	28° C.
<i>Bacillus subtilis</i> .....	extract	176	1	144	80	0	0
<i>Aerobacter aerogenes</i> .....	“	0	0	1	0	0	0
Sterile controls .....	“	158	142		112		139
<i>Aspergillus niger</i> .....	leaf disks	155	2	1	254	2	0
<i>Aspergillus ochraceus</i> .....	“	65	4	25	110	4	0
Sterile controls .....	“	136	78	61	152	67	112

not brought about by pure cultures, and that combinations of certain organisms either may hasten or retard the process of inactivation, depending upon the environmental conditions, such as temperature and oxygen relations. Certainly, it is improbable that such rapid and complete inactivation occurs in nature (1, 4) as may be secured with *A. aerogenes* or *Aspergillus niger* in pure culture under optimal conditions for growth of these organisms.

#### Oxygen Relations

The longevity of *tobacco virus 1* in sterile extract and in dry plant tissues, i.e., in the absence of microbial activity, is readily understandable in view of its inherent characteristics and the important rôle of microorganisms in its inactivation. The long-continued survival of the virus in stored plant extract under conditions of heavy decomposition and putrefaction is not so easily explainable. It has been suspected for some time that the low oxygen supply bore some relation to this survival and that aeration of the virus extract resulted in inactivation (5). The latter type of inactivation may conceivably be due either to direct chemical oxidation or to supplying the flora in the extract with the oxygen necessary for growth and activity. The former hypothesis appears to have been generally accepted as of some significance in inactivation.

A logical test for this assumption may be made by exposing sterile virus extracts to a continuous supply of air under aseptic conditions. An appara-

tus for this purpose was constructed that seemed to serve admirably. Two-arm culture tubes were prepared, which, when set in a slow mechanical rocker, poured the liquid culture medium in a thin layer from one arm to the other without exposure to contamination (Fig. 1). It was found advisable to shut off part of the air supply in the cotton-plugged tubes with caps of tinfoil or cellophane to reduce evaporation to a minimum, when aeration was continued for many days.

Filter-sterile extracts of *tobacco virus 1*, transferred in this manner every two seconds for as long as 28 days, failed to show virus inactivation in all cultures that remained sterile (Table 6). The surprising result of a fairly

TABLE 6.—*The influence of continuous aeration by rocking in two-arm culture tubes on tobacco virus 1 in sterile plant extract*

Experiment	Tested after (days)	Number of lesions on 3 hybrid leaves after			
		Aeration, (at dilution of)		No aeration, (at dilution of)	
		1-10	1-100	1-10	1-100
1	7	270	73	167	11
	14	460	47	165	28
	23	306	22	89	32
2	7	94	31	91	40
	9	202	14	110	13
	15	126		57	
	28	342			
3	8	218	67	222	75
	15	378	72	143	32
Average		266	46	130	33

distinct increase of virus particles in the extract was more conspicuous than was evidence of a decline. Attempts to prove actual multiplication by serial transfers through sterile extract from healthy plants failed, however, and it seems most likely that the continued agitation of the extract breaks up "clumps" of virus particles in the Berkefeld-filtered extracts. At any rate, it seems most improbable, from the data shown, that any direct chemical inactivation of *tobacco virus 1* results from combinations with free oxygen.

If sterile virus extracts aerated in this manner accidentally become contaminated with bacteria or fungi, rapid inactivation may or may not occur, depending upon the species of the contaminant. When the sterile virus extracts are inoculated with pure cultures of known species, the rate of inactivation may be very rapid as, for example, with *Aspergillus niger* or *Aerobacter aerogenes*; or relatively slow, as with *Staphylococcus aureus* or *Bacillus radiobacter* (Table 7). Judging by the growth, this is not entirely a matter of the favorableness of the cultural medium to the organism. Preliminary results also indicate that the nutrients present influence

TABLE 7.—*The inactivation of tobacco virus 1 in continuously aerated extract inoculated with different organisms in pure culture*

Experiment	Organism	Number of lesions on 3 hybrid leaves at 1-10 dilution after aerating		
		4 days	8 days	15 days
1	<i>Bacillus subtilis</i> .....	51	55	96
	<i>Aerobacter aerogenes</i> .....	6	0	0
	<i>Actinomyces scabies</i> .....	98	28	63
	<i>Trichoderma</i> sp. ....	43	7	31
	Sterile controls .....	156	232	277
2	<i>Bacillus radiobacter</i> .....	70	46	
	<i>Sacchromyces</i> sp. (Red Star) .....	83	48	
	<i>Serratia marcescens</i> .....	0	2	
	<i>Proteus vulgaris</i> .....	5	8	
	<i>Serratia keilensis</i> .....	10	0	
	<i>Erwinia carotovora</i> .....	3	0	
	<i>Aspergillus niger</i> .....	0	0	
	Sterile controls .....	340	182	
3	<i>Staphylococcus aureus</i> .....	53	30	
	<i>Phytomonas tumefaciens</i> .....	16	2	
	<i>Phytomonas angulata</i> .....	9	2	
	<i>Rhizoctonia</i> sp. ....	40	1	
	<i>Macrosporium solani</i> .....	17	6	
	<i>Aspergillus ochraceus</i> .....	0	0	
	Sterile controls .....	59	57	

the rate of inactivation of the virus by the microorganisms. The introduction of broth into the plant extract, for example, markedly reduced the rate of inactivation, probably as a consequence of furnishing a more readily available supply of some particular nutrient than exists in the plant extract alone, with the result that the virus is, perhaps, not so rapidly broken down.

Some trials, performed to show the influence of aeration on the comparative rate of inactivation, failed to yield conclusive results; but the trend of such trials with *Aerobacter aerogenes* is indicated in table 8, which shows that the inactivation is more rapid in the aerated than in the nonaerated cultures.

TABLE 8.—*The influence of continuous aeration by rocking in two-arm culture tubes on the inactivation of tobacco virus 1 by aerobacter aerogenes*

Aeration	Number of lesions on 3 hybrid leaves at 1-10 dilution after				
	0 days	1 day	2 days	5 days	7 days
Aerated .....	600	160	156	78	44
Not aerated .....	750	600	500	716	202
Sterile control .....	600			900	

The probable relation of aeration to the relatively rapid rate of inactivation of the virus in sand, as compared to field soil, was previously noted in this laboratory (5). At this time some data were presented showing that

when aseptic conditions are maintained in moist virus-infested sand, little or no inactivation occurs (5). Though the older technique of study was used, the limited data obviously showed the relation of the soil flora to the inactivation of the virus. It was of some interest to again determine the relation of oxygen to the inactivation of the virus in sand and in soil, using the local-lesion method of measurement. Small weighed lots (40 grams) of sand and soil, which had been uniformly infested with virus extract, were placed in small Erlenmeyer flasks. Half of these flasks were placed in an oxygen-free atmosphere and the others were allowed to stand in the ordinary atmosphere but not permitted to become dry. A portion of the data (Table 9) again shows that the virus is only slowly if at all inactivated in sand or soil

TABLE 9.—*The relation of oxygen to the inactivation of tobacco virus 1 in pure sand and in field soil with normal flora present*

Medium	Oxygen	Average number of lesions on 3 hybrid leaves after (days)					
		0	15	30	60	90	120
Soil	+	265	58	82		54	6
	—			850		725	410
Sand	+	530	375	193	81	133	36
	—			900	355	625	425

when oxygen is absent. Though not obvious from the results shown in this table, it has become increasingly evident from scattered data that the more rapid inactivation in moist sand as contrasted to field soil is attributable in large part to the more suitable conditions for aeration in the former, thereby favoring microbial activity.

It has previously been shown that attenuated strains of *tobacco virus 1* may be found in soil where the virus is being inactivated (5). The attenuation in these soils is associated possibly with inactivation and, hence, is due to the activity of the microorganisms. It consequently was of some interest to determine if such attenuated strains were formed in pure cultures. A number of single local lesions from the hybrid host, which had been inoculated with almost completely inactivated cultures, were ground up with water and transferred to ordinary tobacco. The results showed in a rather striking manner that fully 98 per cent of such local lesions yielded typical non-attenuated mosaic. Roughly, 2 per cent of such local lesions yielded attenuated virus, giving milder than ordinary mosaic symptoms on tobacco. Hence, this attenuation was presumably a consequence of the action of pure cultures of the microorganisms used.

#### DISCUSSION

Twenty known species of bacteria and 25 known fungi, together with almost an equal number of undetermined species, were used in this prelimi-

nary survey of the inactivating influence of microorganisms on *tobacco virus 1*. The number of species is not large but is believed to be sufficiently representative to indicate the approximate range of variation in behavior. The experiments were, in part, the outgrowth of earlier studies in this laboratory on the cause and rate of the destruction of this virus in soils and in plant tissues under natural conditions, as bearing on the application of control measures for the ordinary tobacco-mosaic disease (1, 4). There can be no doubt that a wide variety of microorganisms play an important rôle in the inactivation of the virus, and, hence, in checking disease development. We are inclined to believe that much of the inactivation due to the rather obscure effect of "weathering" (4) is also a consequence of microbial activity, since there is no good evidence that freezing, desiccation, or aeration alone can inactivate the virus in plant tissues.

Special interest in these investigations has centered around the nature of the virus itself, as indicated by its reaction to what are perhaps delicate physiological differences between certain microorganisms. No answer can yet be given to many hypotheses that have been developed, but disproval or verification of certain theories as to the actual nature of the virus may possibly be found in more detailed studies of the physiological reaction of certain microorganisms on the virus. Our studies thus far suggest that the inactivation by bacteria or fungi is most likely due to the utilization of the virus constituents in their metabolism. All species are not equally capable of utilizing the particular form of material present, and these either fail to or only slowly inactivate the virus.

Is it likely that the virus material, so readily inactivated by certain organisms, is protein in nature, and that proteolytic enzymes are directly concerned? A suggestion was sought by growing the species used on gelatin media to determine liquefaction. Out of the 20 bacteria used, 6 were distinctly nonliquefiers, and included *Aerobacter aerogenes* and *Bacillus radiobacter*, the 2 organisms that represented the 2 extremes of inactivation on the virus. *Pseudomonas fluorescens* and *Bacillus subtilis*, among the heaviest liquefiers, were not distinctive inactivators. Five fungi, including *Botrytis cinerea* and *Venturia inaequalis*, were nonliquefiers, but were among the best inactivators. *Penicillium baiiolum* and *Aspergillus flavus* proved to be comparatively good liquefiers, but were no better inactivators than the average fungi used. In general, no correlation was found between gelatin liquefaction and virus inactivation. It is, of course, not to be assumed that a virus protein is sufficiently comparable to gelatin protein to justify comparison, but comparisons of this sort suggest possible improvements in methods and technique for the further study of the virus in relation to its composition.

The rapid destruction of the virus by certain microorganisms is not necessarily indicative that the virus is a chemical substance as contrasted to a living organism. Little is yet known about the agents responsible for the

destruction or inactivation of microorganisms in nature or in impure cultures. It is not unlikely that many parasites in particular succumb directly to other organisms, where competition, antagonism, or other unfavorable conditions are now held responsible for their failure to persist or multiply.

#### SUMMARY

The inactivating action of a number of bacteria and fungi in pure culture on the virus of ordinary tobacco mosaic (*tobacco virus 1*), both in leaf tissue and in plant extract, has been investigated.

The bacteria, as a group, are much less effective in destroying the virus in leaf tissue than are the fungi. This result is explained by the low cellulose-decomposing power of the bacteria used, and the consequent prevention of their reaching the virus particles in the cells. The fungi, being good cellulose decomposers, soon obtain direct contact with the virus in the plant cells and are then able to inactivate the virus itself.

Many bacteria, however, readily and rapidly inactivate the virus when in the form of free particles in extract permitting direct contact, although other bacteria are poor inactivators, even under these circumstances. *Aerobacter aerogenes* is, for example, a very rapid inactivator, and *Bacillus radiobacter* or *Phytomonas tumefaciens* are relatively poor inactivators.

The fungi appear to be uniformly good inactivators of the virus. However, a very considerable period of time may be required for complete inactivation by fungi in pure culture, some virus often remaining infectious after 4 months' exposure to the organisms held at room temperatures.

The rate of inactivation by both bacteria and fungi may, in some cases, be greatly increased by aerating the cultures. Complete inactivation in as short a period as 3 days has been secured in aerated cultures.

Continuous mechanical rocking of two-arm culture tubes proved to be a good method for aerating cultures under sterile or pure culture conditions. The continuous aeration (or agitation) of sterile virus extract for as long as 28 days resulted in no inactivation, but rather in a fairly marked increase in the number of local lesions obtained as compared with nonaerated extracts. It seems most likely that the previously noted inactivating action of oxygen on virus extracts resulted from favorable oxygen conditions for microbial activity.

When oxygen is completely excluded from virus-infested moist soil, inactivation of the virus therein is greatly if not completely checked because of the reduced activity of the aerobic organisms in the soil.

Bacteria and fungi in pure cultures occasionally attenuate a small percentage of *tobacco virus 1*. Over 98 per cent of the local lesions tested from cultures in which the virus was almost completely inactivated yielded normal virus upon reinoculation to *Nicotiana tabacum*.

No correlation was found between the efficiency of species of bacteria or fungi as inactivators and their power to liquefy gelatin. It is suggested,

however, that modifications in the methods and technique for determining the influence of microorganisms upon viruses may aid in verifying their true nature.

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U. S. DEPARTMENT OF AGRICULTURE.

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#### PHYTOPATHOLOGICAL NOTES

*Cell Inclusions in Onion-yellow Dwarf*.<sup>1</sup>—In 1935, Tate<sup>2</sup> published a rather unusual discussion of the intracellular abnormalities associated with the yellow dwarf of onions. The difficulty encountered in differentiating the inclusions from nuclei and their close proximity to nuclei, caused him to conclude that "their position with reference to the nucleus of the cell and their frequent close similarity to nuclei suggest that they were of nuclear origin, possibly through amitotic nuclear division." Leaves from onion plants showing typical yellow-dwarf symptoms were given to me by L. D. Leach of Davis, California. Portions of these were fixed in aceto-formalin and processed with the dioxan wholemount technique described by McWhorter and Weier.<sup>3</sup> Examination of this material stained in carbol-fuchsin diluted with equal parts of dioxan shows no unusual structures in the development or appearance of the cell inclusions. They are composed of minute rod-like structures and are enveloped with cytoplasmic trailings. Adjoining nuclei can be distinguished easily by the presence of characteristic reticulate structures that retain stains of the fuchsin group to a greater degree than the composition of cytoplasmic structures permits. These differences are shown in the accompanying photograph made by filtering through a 56 blue-green filter the light transmitted by the red stain and recording the image from a 3 mm. 1.40 N. A. objective on a panatomic film.

<sup>1</sup> Published as Technical Paper No. 266 with the approval of the Director of the Oregon Agricultural Experiment Station. Contribution of Department of Botany.

<sup>2</sup> Tate, H. D. Intracellular abnormalities associated with yellow dwarf of onions. Iowa State Col. Jour. Sci. 9: 677-683. 1935.

<sup>3</sup> McWhorter, F. P. and E. Weier. Possible uses of dioxan in botanical microtechnic. Stain Technol. 11: 107-117. 1936.

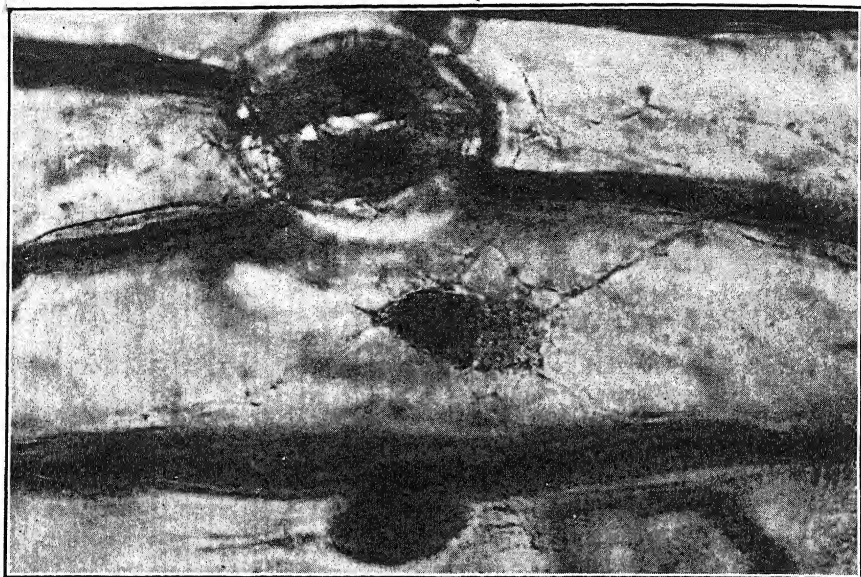
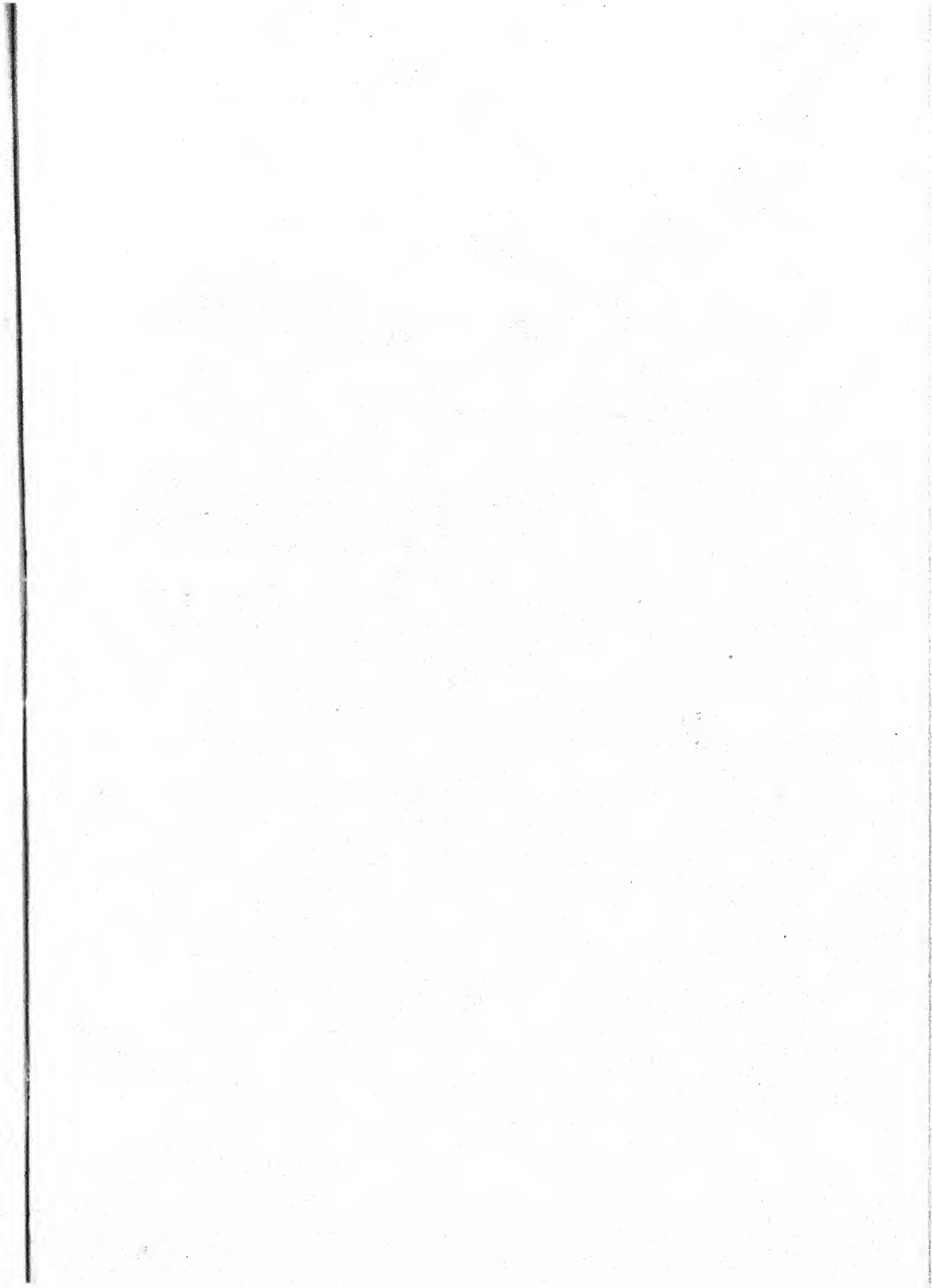


FIG. 1. Cell inclusions in a surface section of an onion leaf from a plant affected with yellow dwarf.

The reticulum of the nucleus and the less definite structures of the inclusion body may be plainly seen.—FRANK P. MCWHORTER, Oregon Agriculture Experiment Station, Corvallis, Oregon.

*Natural Infection of Grasses with Puccinia graminis.*—Susceptibility of species and varieties of grasses to natural infection by *Puccinia graminis* Pers. was studied during the epidemic of wheat stem rust in 1935 at Mandan, North Dakota. *P. graminis* severely damaged 5 species of grass seedlings in nursery rows of *Agropyron inerme* (S. and S.) Rydb., *Agropyron spicatum* (Pursh.) S. and S., *Deschampsia atropurpurea* (Wahl.) Sch., *Elymus condensatus* Presl., and *Poa bulbosa* L. Although these 5 names probably are correct, specimens of these grasses with heads are needed for checking their identifications. The following species produced heads and were abundantly infected with *P. graminis*: *Agropyron pauciflorum* (Sch.) Hitchc., *A. semicostatum* (Steud.) Nees P.I. 101645, *A. smithii* Rydb., *A. strigosum* Coulter, *A. violaceum* (Horn.) Lange, *Avena fatua* L., *Avena sativa* L., *Elymus canadensis* L., *Hordeum jubatum* L., and *H. vulgare* L. One variety of *Agropyron smithii* was nearly immune from *P. graminis*. *Agropyron cristatum* (L.) Beauv. was immune from *P. graminis*. The following species were mildly damaged by *P. graminis*: *Agropyron sibericum* P.I. 101646, *Bromus anomalus* Rupr., *Elymus pseudoagropyron* P.I. 107672, and *Elymus virginicus* L., P.I. 101657.—P. A. YOUNG, Formerly, Soil Conservation Service, U. S. Dept. of Agriculture, Mandan, North Dakota.







ISMÉ ALDYTH HOGGAN

## ISMÉ ALDYTH HOGGAN

1899-1936

JAMES JOHNSON

In the early autumn of 1924, Ismé Aldyth Hoggan came to the University of Wisconsin as a graduate student from Cambridge University. As a young British woman she probably doubted the wisdom of leaving her native land and Cambridge with its medieval traditions, but she soon came to like Madison, its environs and its people. Miss Hoggan finally chose to continue her professional career here and, except for short vacation periods elsewhere in America and brief journeys to Great Britain, remained at Wisconsin.

It was at the suggestion of Dr. E. J. Butler, Director of the Imperial Bureau of Mycology, who had shortly before visited America, that Miss Hoggan came to Madison to pursue her studies with Professor Lewis R. Jones. A mutual feeling of high respect for ability and personality was soon established between teacher and student, which continued through the later years. It was largely through the endeavors of Professor Jones that Miss Hoggan was placed by immigration officials on the preferred quota, permitting immediate admission as a skilled research worker in agricultural science. This decision of the immigration officials established a precedent of considerable international significance for foreign students in agriculture seeking permanent admission to America. Though Dr. Hoggan was still a British subject at the time of her death, she had taken out her first papers and expected soon to become an American citizen.

Ismé Aldyth Hoggan was born, March 23, 1899, in London. Showing early natural ability and inclination as a student, she was given the opportunities of an education far beyond those usually sought by English women. Her preparatory training was taken at the North London Collegiate School for Girls. Here she early showed an aptitude for languages, carrying French, German, Latin, and Greek, along with sciences, especially chemistry, during four years. Her proficiency in chemistry was such that she was urged to continue in this field, but, after two more years of chemistry at Cambridge, she chose botany as the more fascinating for a scientific career. Miss Hoggan was awarded the Bachelor of Arts degree with honors (Natural Sciences Tripos) by Cambridge in 1922. She was then made a research scholar from Newnham College (her own college), and engaged in mycological research at the Botany School of Cambridge under the direction of Professor F. T. Brooks. Here she continued for two years, taking her Master of Science (Contab.) degree, and completing two pieces of research published under the titles "*On Dematium pullulans* de Bary" and "The parasitism of

*Plowrightia ribesia* on the currant." Upon being offered another scholarship, she then elected to come to America for more specialized work in the field of plant pathology. Because of her high standing at Cambridge, she was awarded an Honorary Fellowship upon arrival at Wisconsin.

Her natural ability and basic training impressed her instructors and associates at the University of Wisconsin, and research positions soon became available. She was at first associated with H. H. McKinney of the United States Department of Agriculture, then stationed in Madison. In the autumn of 1925 she came to the writer's laboratory, on a University Research fund grant, to pursue cytological research on certain plant viruses. The evidence along other lines had indicated that the viruses concerned were distinct entities. Miss Hoggan's cytological pictures supported this conclusion. She further showed that the inclusion-bodies (x-bodies and striate material) were characteristic of certain viruses only. With these viruses on different susceptible host species, the inclusion bodies occurred. With other viruses inducing similar gross symptoms on these same hosts, the inclusion bodies were not found. She showed further, for the first time, that these inclusion bodies developed from the cytoplasm and were not essentially foreign bodies in the cells. These facts dealt a severe blow to the then current belief that the so-called x-bodies bore a causal relation to virus diseases. Miss Hoggan's cytological work was marked by unusual excellence of technique and power of interpretation. She was granted the degree of Doctor of Philosophy by the University of Wisconsin in 1927 on the basis of these researches supplementing her earlier work. Thereafter, through cooperative arrangements with the United States Department of Agriculture and her appointment as Agent, it was possible for her to devote her undivided attention to plant virus problems. In 1933 she was appointed Assistant Professor on the Faculty of the University of Wisconsin.

Dr. Hoggan soon became intrigued with the insect transmission of the viruses. In this field she found a congenial type of work with a long-time interest. The classical example of insect transmission of a plant virus usually cited at the time was that of the ordinary tobacco mosaic virus by means of aphids. She showed the erroneous features of this conception, and assembled data on the subject with such care and completeness that it may long remain a model for research on insect transmission. Aphid transmission studies, with especial attention to the ordinary cucumber mosaic virus, were continued over a period of about seven years. Much of our understanding of the aphid relations with this virus is due to Miss Hoggan's work. Her demonstration of selective transmission by aphids from hosts infected with two viruses was highly significant. Indeed, may this not remain one of those obscure phenomena in the virus field that should check us from too confidently theorizing as to the actual nature of a virus.

As a scientist Dr. Hoggan developed and applied the unusual natural abilities she had shown as a student. In her work and in her reasoning she utilized the excellent fundamental background of her earlier training. Hence, she had the judgment to recognize what was significant in a problem, and she wasted little effort on nonessential or vague ideas. This, coupled with the patience and persistence to complete whatever she undertook, enabled her to work with unusual efficiency. Precision, neatness, and reliability were characteristic of all her experiments and her records. She followed the literature in her field thoroughly and critically. When results were prepared for publication, few were her equal either in organization of material or choice of words. Consequently, she was frequently called upon to edit manuscripts of others. National recognition of her merit in this field, through her selection as an associate editor of the journal *PHYTOPATHOLOGY* at the Atlantic City meetings of the American Phytopathological Society, came just too late for her to be apprised of it.

She was affiliated with the following scientific societies: British Mycological Society, The Association of Applied Biologists, The American Phytopathological Society, Sigma Xi, and Sigma Delta Epsilon.

Ismé Hoggan was far from being a cloistered type of scientist; in fact, she felt that research was not her special calling. Her undergraduate interests in literature, art, and languages remained in the background as fields in which she might have derived keener or more enduring satisfactions. Teaching as a profession, however, did not appeal to her; whereas research offered in some measure the freedom of thought and the independence of time she desired. She derived much pleasure from good books, pictures, music, and social contacts with friends. Among her greatest joys was active participation in sports, especially field hockey and ice skating, and at every opportunity she planned hikes in the mountains or life at the seashore. She was especially attracted by scenic beauty, which she carefully sought to record with her camera. All these things she accomplished despite some considerable handicaps in general health, which she fought courageously to the last.

Ismé Hoggan passed away in the early morning of December 28, 1936, and was buried at Forest Hill in Madison, not far from the campus where she worked. Many letters of high tribute to her have come to us from friends and colleagues, particularly from Great Britain. The science of plant pathology has lost a brilliant mind, our laboratory an excellent investigator, and her colleagues a true friend and comrade.

#### LIST OF PUBLICATIONS

1. On *Dematium pullulans* de Bary. Brit. Mycol. Soc. Trans. 9: 100-107. 1923.
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## EFFECT OF BORDEAUX MIXTURE ON THREE VARIETIES OF POTATOES WITH RESPECT TO YIELDS, COMPOSITION OF TUBERS, AND CONTROL OF SCAB

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In a study of the effect of Bordeaux mixture on the growth of potatoes, it was found that the varieties Irish Cobbler, Russet Rural, and Green Mountain responded approximately to the same extent in final increases in yields to the different copper treatments used (14). In connection with this work, an attempt was made to study the effect of Bordeaux mixture on the tuber composition and scab control.

### EXPERIMENTAL PROCEDURES AND METHODS OF ANALYSES

In these experiments the spray was applied at the rate of 100 gals. per acre and at 400 lbs. pressure. The following are the concentrations of Bordeaux mixture used per application: 6-3-50; 10-5-50; 8-4-50; 6-3-50; 4½-2½-50; and 3-1½-50. The total amount of copper sulphate applied amounted to 75 lbs. per A. per season. This schedule follows approximately the growth rate of the potato foliage, giving a more or less equal amount of copper per exposed unit of leaf surface. It was shown earlier that this schedule produced larger yields and better grades of tubers than did schedules calling for equal amounts of copper per application, or for the larger amounts of copper in the latter part of the growing season (10, 11, 12).

The three varieties used in these experiments were planted June 18, 1934. The plots were arranged in accordance with the half-drill-strip method described by Mader and Blodgett (12), giving 4 comparisons for the treated and nontreated of each variety. Plants from the sprayed and nonsprayed blocks of the different varieties were dug at different stages of growth during the season in order to study the effect of Bordeaux mixture on foliage and tuber development. Ten plants per unit were selected and dug in 4 comparable places to give a total of 40 plants for each date of digging for the sprayed and nonsprayed of each variety. The data showing the results are here expressed as average weights per plant. The number of tubers per hill in the sprayed plots of each variety also was recorded throughout the season. The tubers of these plants were separated into different weight classes to show whether Bordeaux mixture affected the rate of tuber enlargement throughout the season. Further, the numbers of healthy and scabby tubers were recorded in each weight class.

During the season tubers of sprayed and nonsprayed plants were intermittently selected at random for chemical analyses. No tubers of less than 5 mm. in diameter were used for the first analyses, and with successive sampling the average tuber size was used. These tubers were thoroughly cleaned, ground to a pulp with a food chopper, and well mixed before sampling. Total nitrogen, reducing sugars, starch, and copper determinations were made on a green-weight basis. Reducing sugars were extracted after the procedure of Appleman and Miller (1). After removal of the alcohol the final determinations were carried out on an aqueous solution by the Munson-Walker method. Total nitrogen was determined by the official Gunning method. The official A. O. A. C. method (2) was used for starch. Copper was determined electrolytically from an ashed sample dissolved in concentrated nitric acid solution.

#### EXPERIMENTAL RESULTS AND DISCUSSION.

The condition of the plants in their early stages of growth are here tabulated.

Sprayed Irish Cobbler and Green Mountain plants showed, in comparison with nonsprayed, a decided retardation in the commencement of anthesis. There was also a smaller number of blossom clusters on the sprayed plants. The response of the 3 varieties with regard to date of tuber setting and rate of enlargement is evident in the above table. The Irish Cobbler tubers were large enough for analysis by August 3, those of Green Mountain by August 17, and those of Rural Russet by September 4.

All sprayed plants, regardless of the variety, showed higher total foliage weights per plant than the nonsprayed plants (Table 2). The greatest differences in foliage weights between sprayed and nonsprayed plants took

TABLE 1.—*Condition of plants in their early stages of growth*

Date	Varieties					
	Irish Cobbler		Green Mountain		Rural Russet <sup>c</sup>	
	A <sup>a</sup>	B <sup>a</sup>	A	B	A	B
July 10	Beginning of anthesis .....		.....	.....	.....	.....
July 24	Anthesis .....	Beginning of anthesis .....	Anthesis .....	Beginning of anthesis .....	.....	.....
	Tubers partially set		Stolons and small tubers present		.....	.....
Aug. 3		Tubers <sup>b</sup>	Stolons increase in length; tubers increase only slightly		Stolons present	
Aug. 17		Tubers		Tubers <sup>b</sup>	Stolons present Very small tubers	
Sept. 4		Tubers		Tubers		Tubers <sup>b</sup>

<sup>a</sup> A nonsprayed; B sprayed.

<sup>b</sup> Tubers large enough for analyses.

<sup>c</sup> Less than 1 per cent of plants in bloom in this variety.

place early in the growing season prior to invasion by insects, chiefly the potato leaf hopper.

In these experiments it was found that sprayed plants wilted more readily than nonsprayed plants. This wilting, which was rather pronounced during the early part of August, lasted about 14 days and occurred between 11 a.m. and 4-5 p.m. Despite this excessive wilting, the increase in foliage weights for this period was larger for the sprayed plants of the varieties Irish Cobbler and Rural Russet. Sprayed Green Mountain plants, however, showed a smaller foliage weight increase for this period than those of the nonsprayed plants. The harmful effect of excessive transpiration on plant growth apparently was demonstrated with the variety Green Mountain, but why the varieties Irish Cobbler and Rural Russet should have behaved differently in this respect is not clear. Judging from the larger foliage weight increases for sprayed plants of the varieties Irish Cobbler and Rural Russet during the period of excessive wilting (early part of August), it appears that application of Bordeaux mixture was not harmful but rather beneficial to plant growth of the 2 varieties. Whether a decrease rather than an increase in transpiration rate occurred from applications of Bordeaux mixture for the above-mentioned varieties, was not determined. The results of Clinton (5) are interesting in this respect. This worker attributes the increase in potato yields in a dry season and the absence of diseases to a decrease in the transpiration rate brought about by application of Bordeaux mixture.



Such workers as Ewert (7), Rumm (17), and Schander (18) also have shown that application of Bordeaux mixture decreased the transpiration rate. On the other hand, an increased rate of transpiration, caused by Bordeaux, was shown by several investigators, Duggar and Bonns (6), Martin and Clark (16), Wilson and Runnels (20, 21, 22).

The apparent contradiction in the rate of transpiration for sprayed and nonsprayed plants, as can be found in the results of above mentioned workers, may be due to differences in conditions at the time the transpiration rate was measured, as well as to the methods employed.

The rate of tuber enlargement early in the season was most rapid in the nonsprayed plants for all 3 varieties under experiment. That of the sprayed plants increased as the season progressed, thus giving a higher total tuber weight per plant for all sprayed plants at the last date of sampling, October 4, for the Irish Cobblers, and October 25 for the Green Mountains and Rural Russets. Similar conditions were reported from other experiments (4, 10, 11) where nonsprayed plants yielded higher tuber weights than sprayed plants in the early stages of tuber enlargement, while, on Long Island, tuber weights of nonsprayed plants remained higher throughout the growing season than those of sprayed plants (9).

The number of tubers per hill dropped as the season advanced, regardless of variety or treatment. The decline was larger for the nonsprayed than for the sprayed plants. This phenomenon of the retention and development of a larger number of tubers in the sprayed plants as against those of the nonsprayed was found in previous experiments (10, 11). Although the average weight per tuber is somewhat larger for the nonsprayed plants in the early stages of tuber development, there is not much difference in the average tuber weights on the last date of sampling (Oct. 25) between the sprayed and nonsprayed plants of the Rural Russets and Green Mountains. Tubers of sprayed Irish Cobbler plants gave a larger average tuber weight on October 4. If the number of tubers per hill and the average weight per tuber be contrasted, it becomes evident that most of the increases in yield from spraying must be due to a larger number of tubers per hill.

Tubers of sprayed plants showed a higher percentage of total nitrogen on the first date of sampling than did those of the nonsprayed plants (Table 2). As tuber weight increased, the percentage of total nitrogen decreased, regardless of variety and treatment. This decrease was more pronounced in tubers of sprayed Green Mountain and Rural Russet plants than in those of the nonsprayed plants, thus giving a high percentage of total nitrogen for the latter on Oct. 4, the last date of analysis. For the Irish Cobblers the percentage of total nitrogen remained higher for the tubers of sprayed plants throughout the entire growing season.

TABLE 2.—*Foliage and tuber weights, number of tubers, and composition of potatoes from sprayed and nonsprayed plants dug at different stages of development*  
(Weight per plant and number of tubers, average of 40 plants. Figures for analysis, average of 4)

Date of sample	Weight per plant in grams		No. of tubers per plant	Weight per tuber in grams	Composition of tubers in grams					
	Tubers				Per 100 grams green weight			Total per plant in tubers		
					Copper <sup>a</sup>	Total nitrogen	Reducing sugar	Starch	Copper <sup>a</sup>	Total nitrogen
Nonsprayed Irish Cobbler										
July 24 .....	141.7									
Aug. 3 .....	222.6	31.2	6.8	20.0	0.249	0.292	0.259	13.875	0.339	0.397
Aug. 17 .....	232.5	136.1	3.5	63.6	0.304	0.282	0.090	14.768	0.677	0.628
Sept. 4 .....	222.6	222.6	2.9	93.9	0.353	0.283	0.034	15.411	0.961	0.770
Sept. 21 .....		272.2								
Oct. 4 .....		277.8	2.9	95.8	0.367	0.280	0.028	15.711	1.020	0.778
Sprayed Irish Cobbler										
July 24 .....	155.7									
Aug. 3 .....	282.1	19.8		15.4	0.989	0.389	0.370	12.843	0.884	0.348
Aug. 17 .....	296.3	89.4	5.8	55.0	0.870	0.374	0.218	14.984	2.393	1.029
Sept. 4 .....	286.3	275.0	5.0	107.5	0.647	0.324	0.072	16.711	2.504	1.254
Sept. 21 .....		387.0	3.6							
Oct. 4 .....		459.3	3.6	127.6	0.585	0.281	0.054	17.625	2.687	1.291
Nonsprayed Green Mountain										
July 24 .....	117.7									
Aug. 3 .....	231.1									
Aug. 17 .....	282.1				0.708	0.351	0.538	9.485	1.216	0.570
Sept. 4 .....	409.7	170.1	6.7	25.4	0.715	0.335	0.282	12.988	2.612	1.015
Sept. 21 .....	428.1	348.7	6.5	53.6	0.749	0.291	0.158	13.233	3.624	1.258
Oct. 4 .....		459.3	6.3	72.9	0.789	0.274	0.017	14.738	4.121	1.325
Oct. 25 .....		521.6	5.4	96.6	0.790	0.254	0.019	15.548		

TABLE 2.—(Continued)

Date of sample	Weight per plant in grams		No. of tubers per plant	Weight per tuber in grams	Composition of tubers in grams					
	Tubers				Per 100 grams green weight			Total per plant in tubers		
	Foliage	Tubers			Copper <sup>a</sup>	Total nitrogen	Reducing sugar	Starch	Copper <sup>a</sup>	Total nitrogen
Sprayed Green Mountain										
July 24 .....	144.6									
Aug. 3 .....	299.1									
Aug. 17 .....	337.4									
Sept. 4 .....	506.1	123.4	8.2	15.0	1.786	0.359	0.312	8.441	1.423	0.415
Sept. 21 .....	541.5	304.8	7.8	39.1	1.153	0.336	0.210	12.354	3.429	0.887
Sept. 21 .....		496.1	8.3	59.8	1.125	0.291	0.182	13.590	5.512	1.270
Oct. 4 .....		737.1	7.8	94.5	1.111	0.256	0.124	15.750	7.400	1.776
Oct. 25 .....					1.004	0.241	0.093	16.178		
Nonsprayed Rural Russet										
July 24 .....	116.2									
Aug. 3 .....	185.7									
Aug. 17 .....	232.5									
Sept. 4 .....	379.9	158.8	7.1	22.4	0.241	0.270	0.189	13.725	0.383	0.429
Sept. 21 .....	411.1	388.4	4.5	86.3	0.311	0.269	0.124	14.750	1.208	1.045
Sept. 21 .....		575.3	4.3	133.8	0.351	0.261	0.041	15.125	2.019	1.502
Oct. 4 .....		623.7	4.3	145.0	0.356	0.260	0.028	16.432	2.220	1.622
Oct. 25 .....										
Sprayed Rural Russet										
July 24 .....	144.6									
Aug. 3 .....	276.5									
Aug. 17 .....	327.5									
Sept. 4 .....	504.6	92.2	10.0	9.2	0.892	0.383	0.296	12.768	0.767	0.353
Sept. 21 .....	569.8	402.6	6.4	62.9	0.570	0.332	0.171	13.452	2.295	1.337
Sept. 21 .....		669.0	6.1	109.7	0.476	0.261	0.065	17.840	3.184	1.746
Oct. 4 .....			5.7	141.8	0.467	0.235	0.062	18.120	3.773	1.899
Oct. 25 .....		808.0								

<sup>a</sup> Copper in milligrams.

In the early stages of tuber enlargement the total nitrogen was higher per plant in the tubers of nonsprayed plants, but greater for those of sprayed plants at maturity. This larger amount of nitrogen per plant in the tubers on the last date of sampling is noteworthy. It may suggest that, on the average, a sprayed plant removes more nitrogen from the soil than a nonsprayed, even though the percentage of nitrogen per tuber may be lower.

The results of analyses of reducing sugars show a higher percentage of sugars for all sprayed Candler and Rural Russet tubers on each date of sampling. The results on the Green Mountain tubers vary.

The percentage of starch was higher for tubers of nonsprayed plants for all 3 varieties in their early stages of enlargement; but, with tuber weight increases, tubers of sprayed plants showed a higher starch content.

All tubers of sprayed plants gave a higher protein-to-starch ratio in their early stages of enlargement than those of the nonsprayed plants (Fig. 1).

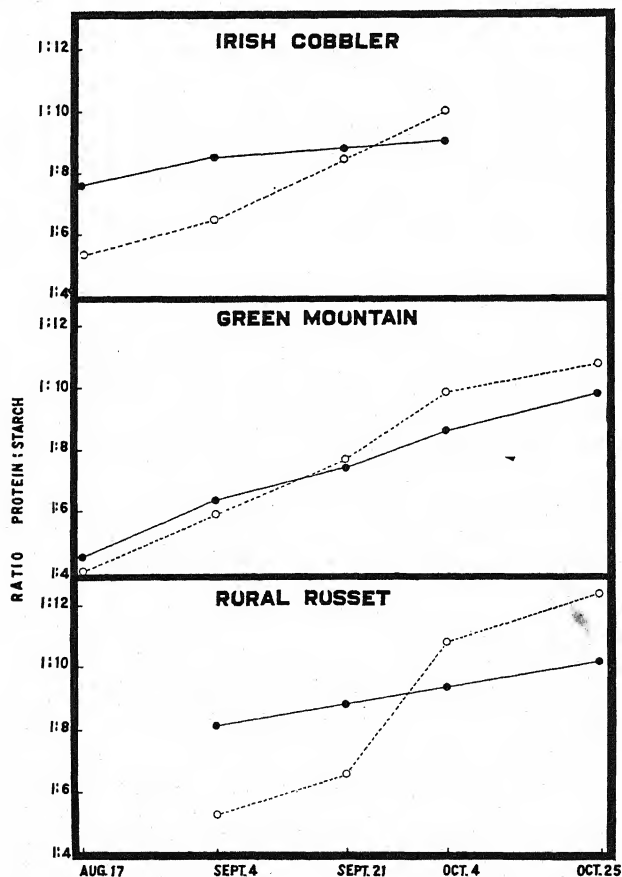


FIG. 1. Ratio of protein to starch of sprayed (open circle) and nonsprayed (solid circle) plants of 3 varieties of potatoes.

As the tuber enlarged, the starch fraction of this ratio increased. This held true for both the sprayed and the nonsprayed tubers. The increase in the starch fraction was more rapid in tubers from sprayed plants, and gave, at tuber maturity, a lower ratio of protein to starch than the corresponding tubers from nonsprayed plants. The lower protein-to-starch ratio of tubers of nonsprayed plants in their early stages of enlargement may aid in explaining why nonsprayed Irish Cobbler and Green Mountain plants bloomed earlier and more profusely than sprayed plants, assuming that the protein-starch ratio plays a rôle in the blooming of plants.

Another noteworthy difference was observed between tubers of sprayed and nonsprayed plants at tuber maturity. The pulp from tubers of the latter darkened more rapidly on standing than did that of tubers from sprayed plants. When whole tubers were cooked it was found that severe blackening occurred in these tubers, but only slight discoloration in those from sprayed plants. Tubers of sprayed plants were, when cooked, also somewhat more mealy than those of nonsprayed plants. The lesser degree of blackening of tubers from sprayed plants was investigated in 1935, and the results showed lower tyrosine and iron content for tubers of sprayed than for those of nonsprayed plants (15).

The total copper content of the tubers was higher for sprayed plants than for nonsprayed ones of all 3 varieties. The Green Mountain variety showed considerably more copper in the tubers than did the other 2 varieties.

When the tubers were harvested a record was made of the number of those belonging to the same weight classes (Table 3). The more rapid rate of enlargement of tubers from nonsprayed plants in the forepart of the period of tuber development is evident. All 3 varieties responded more or less to the same extent. On the last date of harvesting, tubers of sprayed and nonsprayed Rural Russet and Green Mountain plants were found to be nearly all of the same average size. Sprayed Irish Cobbler plants yielded a larger average tuber on the latest date of harvesting than did the nonsprayed ones. The Green Mountain variety tended to set tubers until late in the season; hence it was not grown in the immediate neighborhood of the experiments described here.

A record also was made as to the number of tubers affected with scab (Table 3). It will be noted that tubers of nonsprayed plants showed a higher percentage of scab than did those of the sprayed plants. These findings agree with results recently reported by Mader and Blodgett (13). Closer examination of table 3 will reveal two distinct tendencies: 1. The potato plant, sprayed or not, retained proportionately more healthy than scabby tubers; 2. Scabby and scab-free tubers grew at different rates, the latter growing the more rapidly (Fig. 2). In expressing the relative percentage of scabby tubers for the total number of tubers (scabby and clean),



TABLE 3.—(Continued)

Date of sample	Weight in grams																		Total tubers	
	Below 25		50	75	100	125	150	175	200	225	250	275	300	325	350	375	400	425		450
	to	25	to	to	to	to	to	to	to	to	to	to	to	to	to	to	to	to		to
Sprayed Green Mountain																				
Sept. 4	A	252	50	15	6	3	1												327	
Sept. 4	B	127	38	13	5	3	1												187	
Sept. 21	A	104	85	48	27	15	10	8	3	6	2	1	0	1	1	0	0	0	311	
Sept. 21	B	24	27	5	4	1	1	1	0	1	0	0	0	0	0	0	0	0	64	
Oct. 4	A	89	76	54	48	24	16	6	3	4	1	2	1	1	2	2			330	
Oct. 4	B	10	10	13	12	1	1	0	1	0	0	0	0	0	0	0	0	0	48	
Oct. 25	A	43	48	46	40	38	34	22	8	6	5	6	4	2	0	2	1	2	311	
Oct. 25	B	0	10	11	10	8	3	2	1	1	0	0	0	0	0	0	0	0	46	
Nonsprayed Rural Russet																				
Sept. 4	A	159	58	40	22	5													284	
Sept. 4	B	84	40	32	20	5													181	
Sept. 21	A	8	23	38	48	30	15	10	4	2	1	1							180	
Sept. 21	B	8	18	20	10	4	1	1	0	0	0	0							62	
Oct. 4	A	7	19	26	36	32	18	10	11	7	3	1	1	1					172	
Oct. 4	B	6	17	20	8	5	2	0	1	1	0	0	0	0					60	
Oct. 25	A	0	5	12	20	24	28	27	24	18	5	4	1	1	1				170	
Oct. 25	B	0	3	12	18	20	4	1	1	1	0	0	0	0	0	0	0	0	60	
Sprayed Rural Russet																				
Sept. 4	A	273	104	14	6														397	
Sept. 4	B	75	42	8	4														129	
Sept. 21	A	20	36	58	60	38	19	15	4	2	1	2							255	
Sept. 21	B	8	9	8	6	4	1	0	0	0	0	0							36	
Oct. 4	A	16	36	42	59	48	21	8	6	2	3	1							242	
Oct. 4	B	2	2	3	3	2	1	0	0	0	0	0							13	
Oct. 25	A	4	19	22	27	29	36	34	32	12	5	2	2	0	2	0	0	0	226	
Oct. 25	B	0	2	3	3	1	1	1	1	0	0	0	0	0	0	0	0	0	12	

a Total number of tubers (healthy and scabby).

b Scabby tubers.

figure 2 shows another point of interest. It seems that in the early stages of tuber enlargement the largest tubers are more susceptible to scab infection than are the smaller ones. This, however, does not exclude the possibility that infection took place earlier in the season on the larger tubers than on the smaller ones, giving the scab more time to develop and resulting in the higher percentage of scab in the largest tuber weight classes. Whether this difference in rate of enlargement between scabby and clean tubers holds in all instances of scab infection, remains to be proved. It may apply only in cases where infection takes place in the early part of the growing season.

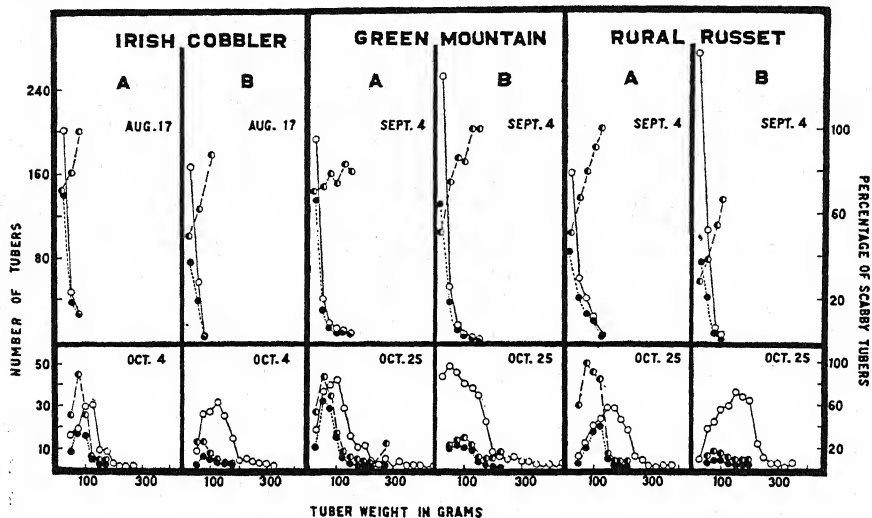


FIG. 2. Tuber of nonsprayed (A) and sprayed (B) plants at 2 dates of sampling showing: open circle, frequency distribution of total number of tubers per 40 plants; solid circle, scabby tubers; and half-solid circles, percentage of tubers infected with scab.

The decrease in scabby tubers in these experiments, as the season advanced, suggests that infection must have taken place in the early part of tuber enlargement. Failure to find any milliped injury on any of the tubers, makes it improbable that the decreased percentage of scab lesions was caused by their feeding activities in eating out the lesions.

The nature of scab resistance of the tubers of sprayed plants is not yet understood. That the increased rate of transpiration of sprayed plants may accelerate their "hardening" and make the tubers less susceptible to scab infection has been suggested (13). It is possible that the relatively high copper and nitrogen and the lower starch content of tubers of sprayed plants in the forepart of the season plays an important rôle in the difference in degree of susceptibility to scab. As to the possibility of a higher copper content being partially responsible for less scab infection, it may be noted that this view is strengthened by the work of van Schreven (19), who



believes that the root system of plants weakened by copper deficiency are more sensitive to *Pythium* and other root parasites. The further fact that plants were grown in a soil of pH 7.5, and copper deficiency occurs more frequently in alkaline than in acid soils, suggest the possibility that differences in the amount of copper in the tuber may partially explain the lesser degree of infection on tubers of sprayed plants. The relatively high amount of copper in tubers of the variety Green Mountain against the lower copper content of tubers of the Rural Russet and Irish Cobbler, with equal degree of scab infection, would, however, contradict such a view.

There is another possibility that may account for the reduction of scab on tubers of sprayed plants. During the spraying operations, especially when the plants are small, much of the spray falls on the soil. The repeated applications of Bordeaux mixture will soon increase the copper content of the soil surrounding the root system. This soil-borne copper, rather than that applied to the foliage, may be responsible for the reduction of scab. This assumption is supported by the work of Kinney (8) and Beach (3), who reported decreases in scab infection by applying either Bordeaux mixture or copper sulphate to the soil at planting time.

#### CONCLUSIONS

Applications of copper in the form of Bordeaux mixture retarded the blooming of Irish Cobbler and Green Mountain plants.

The tuber and foliage weights of sprayed Irish Cobbler, Green Mountain, and Rural Russet plants were larger than those of nonsprayed plants.

All sprayed plants retained more tubers per plant than nonsprayed plants of the same variety.

All varieties showed evidence of retardation in tuber enlargement because of spraying in the forepart of the period of tuber development.

Rural Russet and Green Mountain differed little in average weight per tuber from sprayed and nonsprayed plants at the final date of harvesting, while, for the Irish Cobbler, the average weight was higher for the sprayed plants.

Tubers of sprayed plants, regardless of variety, showed a marked reduction in the percentage of scab.

In the early stages of tuber enlargement, the largest tubers of sprayed and nonsprayed plants showed a greater percentage of scab infection than those of the smaller tubers.

Scabby tubers developed more slowly than healthy ones, thus giving, at maturity, a greater percentage of scabby tubers in the smaller weight classes.

Tubers from sprayed plants had a higher copper content than those of the nonsprayed plants.

The percentage of total nitrogen was higher for tubers of sprayed plants in the early stages of development, but, at maturity, the percentage of total nitrogen was higher in tubers of nonsprayed plants.

The total nitrogen in tubers expressed per plant was higher for those of sprayed plants of all varieties under experiment.

Tubers of sprayed plants showed higher reducing sugar than those of nonsprayed plants.

While the starch content was higher for tubers of nonsprayed plants in the early stages of tuber enlargement, it was higher for those of sprayed plants at maturity.

The ratio of protein to starch was higher for tubers of sprayed plants in the beginning of tuber enlargement, but lower at maturity, thus giving a lower ratio of protein to starch than corresponding tubers of nonsprayed plants.

Tubers of sprayed plants darkened less on cooking and were somewhat more mealy than those from nonsprayed plants.

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## RELATION OF LIVESTOCK TO THE CONTROL OF SCLEROTINOSIS OF LETTUCE

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(Accepted for publication August 23, 1937)

Livestock may play a double rôle in the control of lettuce sclerotinosis. Stock fed on cull lettuce and trimmings in corrals and barnyards on the farm necessitate the hauling thither of the lettuce refuse from the packing sheds, a practice that seldom fails to spread sclerotinosis through the agency of the abundant sclerotia of *Sclerotinia* present in the scattered plant parts and in manure. On the other hand, livestock materially aids in the control of sclerotinosis when the animals are utilized to clean infected lettuce fields after the crop is harvested. This statement applies particularly to sheep. In the process of cutting lettuce, noticeably diseased heads are left in the field. The discarded and rejected infected heads continue to produce large numbers of sclerotia on the surface of the ground or beneath, even when they are more or less covered with soil by subsequent plowing; but, after sheep have been pastured in the field for a time, such refuse is consumed and the development of sclerotia consequently ceases.

Sclerotinosis of head lettuce, caused by *Sclerotinia sclerotiorum* (Lib.) Mass., has resulted in heavy losses in the Salt River Valley of Arizona<sup>2</sup> where the disease is favored by environmental conditions during the major part of the lettuce season. Lettuce is there an irrigated fall, winter, and spring crop. The seed for the fall crop is planted in the field in early September. Temperatures remain too high for *Sclerotinia* until the middle or last of November, when the heads are approaching maturity; therefore, the young

<sup>1</sup> Acknowledgments are due Mr. M. F. Wharton, formerly Associate Horticulturist of the Arizona Agricultural Experiment Station, for field observations in connection with the pasturage of sheep in harvested lettuce farms; also to Mr. Donald J. Smith, Assistant Plant Pathologist, and Mr. Manny Gottlieb, Research Assistant, for their help with the feeding experiments.

<sup>2</sup> Brown, J. G., and K. D. Butler. Sclerotiniosis of lettuce in Arizona. Arizona Agr. Expt. Sta. Tech. Bull. 63. 1936.

plants in the first crop escape the disease and loss is restricted to the mature plants. Subsequent plantings of lettuce in cool weather show sclerotinosis in all stages of development, from the late seedling to mature heads. Finally, the warm weather of April checks the activity of the fungus, and in the latest planted fields, the loss may be greatest in the younger seedling and half-grown stages. Even the late crops, however, have shown considerable loss from sclerotinosis. As a result, some of the most severely infected fields have been withdrawn from lettuce production and have then been used for wheat or other nonsusceptible crop, and alfalfa land has been broken to replace the acreage for lettuce.

The use of alfalfa fields for lettuce afforded the occasion for the studies here briefly reported. Alfalfa in Arizona never has been attacked by *Sclerotinia sclerotiorum*, so far as known. Yet, in the first crop of lettuce in a field that had been continuously used for alfalfa for 3 years and in another field previously in alfalfa for 7 consecutive years, sclerotinized lettuce heads occurred here and there throughout the planting in the 1936-37 crop. None of the heads showed signs of aerial infection, but they appeared to have been attacked from the soil. The evidence suggested that in some way sclerotia had been carried into the alfalfa fields. Furthermore, the irregular distribution of the infected lettuce heads, rather than the occurrence of greater numbers near the entrance gate, suggested that sclerotia might have been deposited in animal droppings. Suspicion was supported by the facts that farm machinery (tractors, cultivators, plows), which might conceivably carry sclerotia, is not interchangeably and contemporaneously used in the two different crops, and that livestock pastured in lettuce fields is frequently turned into alfalfa fields when the lettuce refuse in the former is consumed. To determine the relation, if any, between the animals and the distribution of sclerotinosis, feeding experiments were conducted on the Rillito Valley Farm of the University of Arizona, 50 miles distant from the nearest infected district.

In preparation for the feeding tests, a small truckload of lettuce infected with *Sclerotinia sclerotiorum* was collected in the Salt River Valley, brought to the laboratory, and used for the growing of sclerotia. From the lettuce approximately 16,000 sclerotia were obtained, carefully washed and air-dried, counted out in lots containing equal numbers, and placed in vials. The sclerotia were fed (usually twice daily) to 3 sheep, separately stalled on a clean, new concrete floor, and supplied with individual feeding basins. In feeding, the sclerotia were mixed either with chopped cull lettuce or chopped green alfalfa. As indicated in the following table, careful counts were made of the numbers of sclerotia eaten by the sheep and the numbers of whole sclerotia that appeared in the faeces.

Records of the feeding of the sclerotia as presented in the table show that sheep No. 1 consumed 6,728 sclerotia and evacuated 62 whole ones, or

TABLE 1.—Summary of sheep-feeding experiment in which lettuce infected with *Sclerotinia sclerotiorum* was fed to three sheep kept under observation 15 days

Date, May	Time since last feeding:		Total elapsed time since feed- ing started:		Sheep No. 1		Sheep No. 2		Sheep No. 3		Total whole sclero- tina elim- inated
	Hrs.	Approx. days	Hrs.	Approx. days	No. sclero- tina eaten	Sclero- tina elim- inated whole	No. sclero- tina eaten	Sclero- tina elim- inated whole	No. sclero- tina eaten	Sclero- tina elim- inated whole	
1	17½	2½	17½	2½	59	0	0	0	10	0	0
1-3	49½	2	66½	2¾	84	0	0	0	61	0	0
3-4	7½	1	74½	3	582	0	494	1	89	3	4
4-5	16½	1	90½	3½	405	2	501	0	344	10	12
5	23½	1	114	4½	76	3	37	2	0	1	6
5-6	63½	1	120½	5	467	10	619	2	25	1	13
6	18	1	138½	5½	587	1	639	0	0	1	2
6-7	53½	1	144½	6	630	3	643	1	0	1	5
7	18½	1	162½	6½	459	1	424	0	154	0	1
7-8	6	1	168½	7	623	0	641	0	636	0	0
8	19½	1	188	7½	603	0	498	1	0	2	3
8-9	53½	1	193½	8	598	6	641	6	585	0	12
9	18	1	211½	8½	638	2	167	1	138	13	16
9-10	61½	1	217½	9	396	5	553	5	644	9	19
10	17	1	234½	9½	521	5	206	6	282	26	37
10-11	23½	1	241½	10	13	3	3	3	32	32	48
11	41½	1½	259½	10½	3	3	1	1	12	12	16
11-12	43½	2	266½	11	4	4	4	4	34	34	42
12	67½	2½	285	12	285	3	0	0	9	9	12
12-13	71½	3	289½	12½	1	1	0	0	4	4	5
13	90½	3½	308½	12½	0	0	0	0	0	0	0
13-14	94½	4	312½	13	0	0	0	0	2	2	2
14	114½	4½	332½	13½	0	0	0	0	0	0	0
14-15	119½	5	337½	14	0	0	0	0	0	0	0
15	138½	5½	356	14½	0	0	0	0	0	0	0
	144½	6	362½	15	0	0	1@	34	0	0	1
			Totals		6728	62	6177	34	3018	160	256
									15923		

a Last feeding of sclerotia. @ Lifeless.

slightly less than 1 per cent of the sclerotia eaten. Of the sclerotia evacuated, 25 were passed in the faeces during the first 3 days after the feeding of sclerotia was discontinued; thereafter, no more were passed. Sheep No. 2 ate 6,177 sclerotia and evacuated 34 undigested whole ones, which represents approximately one-half of 1 per cent of the sclerotia devoured by this animal; 14 sclerotia appeared within 2 days after the feeding of the sclerotia ceased and 1 appeared on the sixth day, but it was lifeless. Sheep No. 3 consumed 3,018 sclerotia and evacuated 160 (about 5 per cent) apparently uninjured sclerotia, of which 119 appeared within 4 days after the feeding of sclerotia ceased; thereafter this animal passed no more sclerotia. (Fig. 1.)

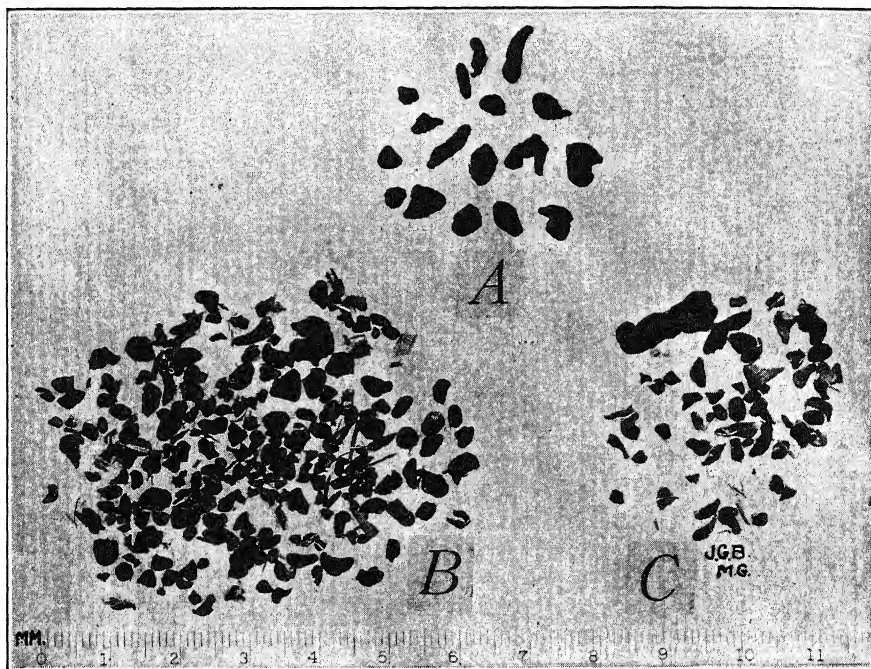


FIG. 1. Sclerotia and pieces of sclerotia of *Sclerotinia sclerotiorum*. A. Normal, representative of the lot fed to sheep. B. Recovered from faeces of sheep No. 3, collected at 5 p. m., May 9, 1937. C. Recovered from faeces of sheep No. 1, collected at 10:30 a. m., May 9, 1937.

The sclerotia evacuated by the sheep in the feeding experiments were tested for viability in two ways: (a) Some were plated on potato—2 per cent dextrose agar and on moist sterile sand, and thereafter compared with similarly cultured sclerotia from the same original lot, but that had not been fed to sheep. (b) Other sclerotia, entire and in large fragments, evacuated in the faeces, were sectioned and treated with Luyet's<sup>3</sup> vital stain; after-

<sup>3</sup> Luyet, B. J. Differential staining for living and dead cells. *Science* (n. s.) 85: 106. 1937.

ward, the sections were compared with sections of sclerotia, both natural and evacuated in faeces, that had grown in culture. The living cells stained cerise red, dead cells yellow or orange, and dying cells an intermediate color. The formula for the stain calls for distilled water solvent, but tapwater was superior in these tests.

Viability of the ingested sclerotia, determined by culture and comparative staining, was low. Of 312 whole and broken sclerotia evacuated in faeces, 3, or 0.99 per cent, produced mycelia; 73 control sclerotia, obtained from the same original lot, but not fed to sheep, contained 44 (60 per cent) that produced mycelia in culture. Besides the evacuated sclerotia tested as above described, 40 additional evacuated whole sclerotia and 60 broken ones were soaked for a short time in a very weak aqueous solution of sodium hydroxide, in order to determine whether they were merely inactivated by the hydrochloric acid of the gastric juice of the sheep, or actually devitalized; none of these produced mycelium. Neither the evacuated sclerotia nor the controls developed apothecia.

#### DISCUSSION

In view of the results obtained by Leach and Mead<sup>4</sup> of California, in feeding the sclerotia of *Sclerotium rolfsii* to sheep, the experiments of the writer are somewhat surprising. The sclerotia of *S. rolfsii*, of course, are much smaller than those of *Sclerotinia sclerotiorum*, and they are considered as less resistant to adverse conditions. However, such conditions obviously do not include subjection to mastication and the action of the digestive juices of sheep. From 10 to 22 per cent of the sclerotia of *Sclerotium rolfsii* consumed by sheep in the California experiments were recovered in a whole condition as compared with 1 to 5 per cent in the case of the sclerotia of *S. sclerotiorum*; viability of the former was 1.6 per cent, and for the latter less than 1 per cent. Possibly the larger size of the sclerotia of *S. sclerotiorum* rendered them easier to break in the process of mastication and thus more readily attacked by digestive enzymes.

To this destruction of the sclerotia in the digestion of lettuce by sheep, as indicated in the feeding experiments, must be added the advantage in control measures entailed by the digestion of vast numbers of potential sclerotia in the mycelia of infected heads. A single head of lettuce produced 212 sclerotia of *Sclerotinia sclerotiorum* and 6 infected heads of cabbage (a crop also attacked by *Sclerotinia* in the Salt River Valley and adapted to the same sheep-feeding practice) developed 2,221 sclerotia in 19 days. On the other hand, the possible spreading of sclerotinosis by sheep through sclerotia evacuated in the faeces can not be ignored. Even 1 per cent of the large numbers of sclerotia ingested by the animals and afterward evacuated

<sup>4</sup> Leach, L. D., and S. W. Mead. Viability of sclerotia of *Sclerotium rolfsii* after passage through the digestive tract of cattle and sheep. Jour. Agr. Res. [U. S.] 53: 519-526. 1936.

in a living condition constitutes a serious source of infection. The feeding trials suggest, however, that this danger of infection easily can be overcome by holding in corrals or on land not intended for susceptible crops livestock that has fed on sclerotinosed plants. The period of 4 days for the quarantine of animals is indicated as safe by the experiments. The importance of quarantine becomes further emphasized when the long period of viability (up to 11 years) of the sclerotia is considered.

Results of the feeding experiments herein described do not prove that the diseased lettuce fields on former alfalfa land were infected by sclerotia that passed through the digestive tract of livestock, but they appear to demonstrate the possibility of such infection. Together with the extensive destruction of the sclerotia of *Sclerotinia sclerotiorum*, the information thus obtained is regarded as an important contribution to the subject of the control of lettuce sclerotinosis.

Incidentally, the vital stain used by Luyet with different plant parts gave such reliable results in testing the viability of the sclerotia of *Sclerotinia sclerotiorum* that it should be tried on other fungi, as well as on the tissues of host plants. The procedure is simple and not time-consuming.

#### SUMMARY

Livestock may prove either baneful or beneficial in relation to the control of lettuce sclerotinosis caused by *Sclerotinia sclerotiorum*, depending upon the manner of feeding. Farm animals, fed on lettuce refuse in corrals and barnyards, distribute the disease; pastured on infected lettuce fields after harvest, livestock, especially sheep, may be useful in the control of sclerotinosis.

Sclerotinosis in the first crop of lettuce on land previously in alfalfa for several consecutive years appeared to attack the plants only from the soil. The irregular distribution of diseased plants and other attendant circumstances suggested infection from sclerotia in animal droppings. Feeding experiments, therefore, were made to determine whether living sclerotia of *Sclerotinia sclerotiorum* can pass through the alimentary tract of sheep.

Sheep, fed approximately 16,000 sclerotia of *Sclerotinia sclerotiorum*, digested to a varying degree 95 to 99.5 per cent of the sclerotia eaten, and they evacuated in a whole condition 1.6 per cent of the sclerotia consumed. Less than 1 per cent of the evacuated sclerotia were capable of growth.

The maximum period for the evacuation of living sclerotia by sheep, as indicated by the feeding experiments, was 4 days; a quarantine period of 4 days is recommended for sheep that have pastured on sclerotinosed fields.

Attention is directed to the value of the vital stain of Luyet, when it is properly checked, for determining the viability of sclerotia of *Sclerotinia sclerotiorum*.



## VEIN-MOSAIC VIRUS OF RED CLOVER

H. T. OSBORN

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Recent studies have demonstrated that there are several distinct viruses capable of producing diseases in legumes. In the course of experiments with two of these viruses (2, 3), a virus disease of red clover, *Trifolium pratense* L., was observed that appeared to be different from any previously reported. Studies were, therefore, undertaken to determine the host range, properties, and method of transmission of the causal virus. Since the virus produces a yellowing of the veins in red clover, it will be referred to as the vein-mosaic virus of red clover.

### MATERIALS AND METHODS

Vein-mosaic virus of red clover was obtained originally from red-clover plants naturally infected in a field near Princeton, New Jersey. The plants were transplanted from the field to a greenhouse. Several passages to seedling red-clover plants by means of the pea aphid revealed the fact that more than one virus was present. Some plants showed mottled symptoms similar to those described for pea virus 2, while others exhibited symptoms of disease along the veins. Successive passages to healthy clover plants from plants showing only vein symptoms served to isolate a virus that produced only the vein-mosaic symptom complex. In order that the virus might be available at all times, it was maintained continuously in a greenhouse by successive transfers to red clover by inoculation with pea aphids and by mechanical inoculation to *Vicia faba* L. plants. Transmission by pea aphids was accomplished in screened cages as described for the transmission of pea virus 2 (3). The mechanical inoculation method used was to rub leaves that had been dusted with carborundum powder No. 320 with bandage gauze that had been dipped in juice containing the virus.

### HOST RANGE AND DESCRIPTION OF THE DISEASE

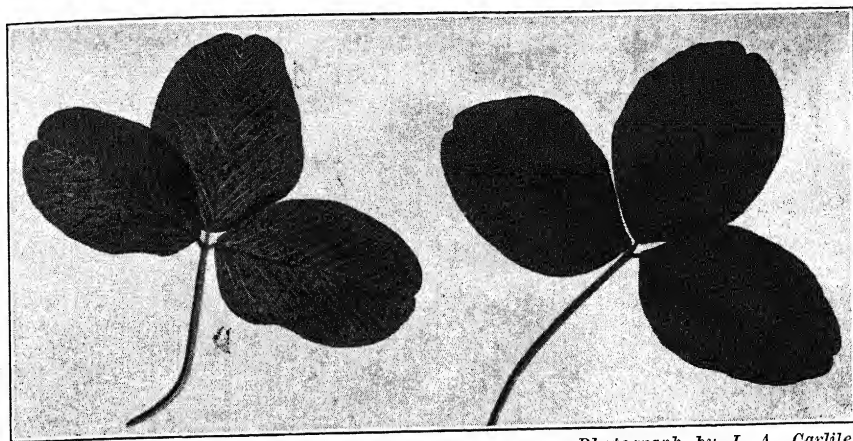
The following host plants were infected with red clover vein-mosaic virus by using the pea aphid as vector: broad bean (*Vicia faba*), Olympia sweet pea (*Lathyrus odoratus* L.), red clover (*Trifolium pratense*), white clover (*T. repens* L.), alsike clover (*T. hybridum* L.), and crimson clover (*T. incarnatum*). The virus was transmitted mechanically from red clover to *V. faba* and from *V. faba* to red clover, white clover, alsike clover, crimson clover, white sweet clover (*Melilotus alba* Desr.), Canada white field pea (*Pisum sativum* L. var. *arvense* Poir.), and garden pea, (*P. sativum*) of the varieties Alderman, Telephone, Perfection, and Horal. The results were

checked in most instances by mechanical transmission from each of the affected hosts to healthy plants of *V. faba*. In the case of garden pea varieties, however, the results were checked in this manner only for the Alderman and Perfection varieties.

No infections have yet been obtained by mechanical inoculation to garden bean (*Phaseolus vulgaris* L.) of the varieties Green Stringless Refugee, Corbett Refugee, Robust, and Great Northern Idaho No. 1, alfalfa (*Medicago sativa* L.), mung bean (*Phaseolus aureus* Roxb.), Turkish tobacco (*Nicotiana tabacum* L.), *N. glutinosa* L., *N. sylvestris* Spegaz. and Comes, *N. rustica* L., and *N. langsdorffii* Weinm. Inoculation to seedling potato plants (*Solanum tuberosum* L.), to plants of the potato variety Green Mountain, and to tomato plants (*Lycopersicon esculentum* Mill.) variety Bonny Best, both by means of the potato aphid, *Macrosiphum gei* Koch, and by mechanical methods, likewise failed to produce the disease. Subinoculations were made to *Vicia faba* from plants that failed to become infected but in no case was the disease produced in the *V. faba* plants.

Symptoms in red clover have been observed within 14 days after inoculation by colonies of pea aphids. Usually, however, the first symptoms appear in from 3 to 4 weeks, and in some cases not until 6 weeks or more, after inoculation. The most conspicuous symptom is development of a yellow color along the veins. Extent and intensity of the yellow color vary widely. It is usually most conspicuous in leaves of new growth, in plants that have been cut back. Sometimes small yellow spots have appeared in interveinal areas, but there is no mottling such as described for red clover plants infected with pea virus 2 (3). The disease causes little or no stunting of plants growing in a greenhouse, the plants having continued to grow without noticeable injury for periods of more than 2 years. A leaf from a diseased plant showing conspicuous symptoms is shown with a leaf from a healthy plant in figure 1. The photograph was taken 114 days after inoculation. Alsike clover, white clover, and white sweet clover, likewise, show symptoms only along the veins and continue to grow with little apparent injury. Crimson clover, on the other hand, is severely stunted, and infected plants die.

In *Vicia faba*, first symptoms of the disease usually appear in from 14 to 21 days, though a longer period sometimes is required. Local necrotic splotches and rings sometimes appear on mechanically inoculated leaves 10 to 12 days after inoculation. In early stages of systematic infection there is a clearing of veins. Later a slight distortion of the tissue on the under surface of infected leaves gives the appearance of whitish bands along the veins. The under surface of a diseased *V. faba* leaf is illustrated in figure 2. A purplish discoloration develops on the surface of the stalks. Diseased plants become badly stunted, and small clumps or rosettes are frequently produced at the base of the stalks. Many diseased plants wilt and die back when growing in a greenhouse.



Photograph by J. A. Carlile.

FIG. 1. Red clover leaves: left, from plant infected by the vein-mosaic virus of red clover; right, from healthy plant.

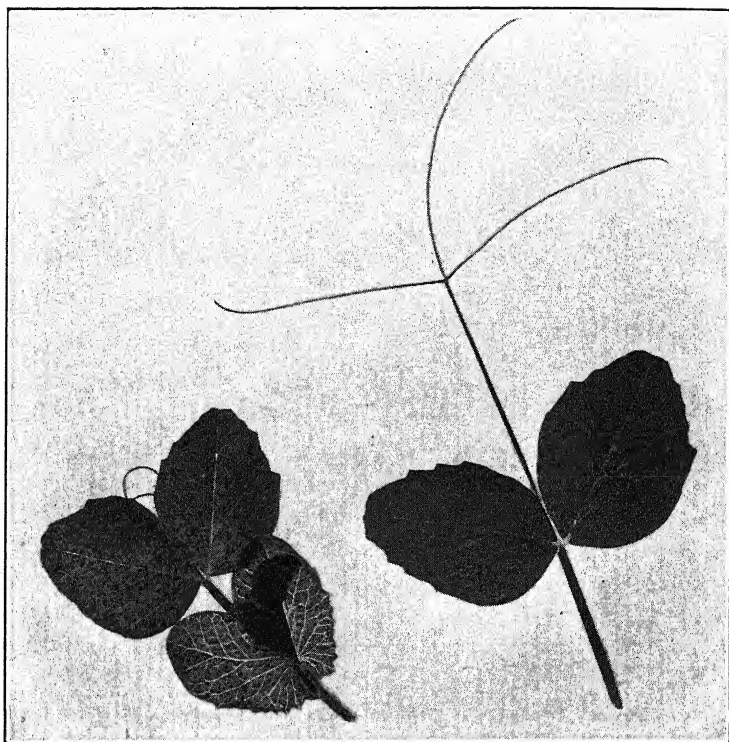
In garden pea plants, clearing of veins usually appears in from 12 to 14 days after inoculation with red clover vein-mosaic virus. The disease produces rosetting of the tip leaves, followed by a rapid wilting, and plants are frequently dead 3 weeks after inoculation. Distinct vein clearing is shown in a leaf from a diseased plant of the garden pea variety Perfection in figure 3. Symptoms in Canada white field pea are similar to those produced in garden pea. Only a few sweet pea plants have been inoculated.



Photograph by J. A. Carlile.

FIG. 2. *Vicia faba* leaves: left, from plant infected by vein-mosaic virus of red clover; right from healthy plant.

A pronounced clearing of the veins was followed by wilting and death of the plants.



Photograph by J. A. Carlile.

FIG. 3. Garden pea leaves of the variety Perfection: left, from plant infected by vein-mosaic virus of red clover; right, from healthy plant.

#### EXPERIMENTS

*Thermal Inactivation and Resistance to Aging in vitro.*—Since the properties of resistance to heat and aging *in vitro* are frequently used as means of classifying or differentiating plant viruses, studies were made to determine these properties for the vein-mosaic virus of red clover. Resistance to heating was determined by placing 2 cc. of undiluted extract from diseased *Vicia faba* plants in thin-wall test-tubes, heating in a water bath at the desired temperature for 10 minutes, and then testing for infectivity by mechanical inoculation to *V. faba* plants. To determine resistance to aging *in vitro*, test-tubes each containing 2 cc. of expressed juice from diseased plants were held for the required period in a laboratory room. The juice was then tested for infectivity by mechanical inoculation to *V. faba* plants. The results obtained are given in tables 1 and 2. The specific vein-

TABLE 1.—*Thermal inactivation of the vein-mosaic virus of red clover*

Temperature (degrees C.)	Plants inoculated	Plants infected
Control .....	60	39
52 .....	10	4
54 .....	10	6
56 .....	10	3
58 .....	10	1
60 .....	20	0

TABLE 2.—*Resistance to aging in vitro by the vein-mosaic virus of red clover*

Time aged	Plants inoculated	Plants infected
15 minutes .....	30	20
24 hours .....	10	4
2 days .....	20	4
3 days .....	20	0
4 days .....	10	0
5 days .....	10	0
7 days .....	10	0

mosaic virus was found to be active after heating for 10 minutes at various temperatures up to and including 58° C., but was inactivated after heating to 60° C. The virus was found to be active after aging *in vitro* for various periods up to and including 2 days, but was inactivated after 3 days' aging.

*Transmission of the Virus by the Pea Aphid.*—Having observed in preliminary tests that the pea aphid was capable of transmitting the vein-mosaic virus of red clover, some experiments were conducted to determine the method of transmission. In one such experiment, pea aphids were fed for 2 days on diseased red clover plants and then 20 of them were transferred to each of 10 fresh red-clover and 10 crimson-clover plants. They were held on these plants for 24 hours and then transferred to a second set of 10 red-clover and 10 crimson-clover plants, where they fed for 3 days. In this experiment, 4 of the red-clover and 7 of the crimson-clover plants exposed to aphids immediately after removal from diseased plants became infected, while all of the plants of the second set remained healthy. This experiment demonstrated that the pea aphid transmits the vein-mosaic virus of red clover to crimson clover as well as to red clover, and that the virus is lost by the aphids when fed for 24 hours on healthy plants.

In another experiment, pea aphids were fed for 3 hours on diseased red-clover plants and then approximately 100 were transferred to each of 10 *Vicia faba* plants, 5 red-clover plants, 2 crimson-clover plants, and 1 alsike-clover plant. At the end of 24 hours they were transferred to a second set of 5 *V. faba* plants and 2 crimson-clover plants. Of the first set of plants exposed, 1 *V. faba* plant, 2 red-clover plants, 2 crimson-clover plants, and 1

alsike-clover plant became infected. Of the second set of plants, all remained healthy. The results show that the aphids acquired the virus in a feeding period of 3 hours on diseased plants and again demonstrated that the virus was lost by the aphids when they fed for a period of 24 hours on healthy plants.

To determine whether there is in the pea aphid an incubation period of the vein-mosaic virus of red clover, large numbers of nymphs and adults were fed for 1 hour on 5 diseased *Vicia faba* plants. The aphids were then removed and 100 were placed on each of 5 healthy *V. faba* plants, where they again fed for 1 hour. They were then transferred to a succession of healthy *V. faba* plants at intervals of several days for a total period of 14 days. The results of this experiment are shown in table 3. Two of the plants

TABLE 3.—Infections obtained with pea aphids when fed for 1 hour on diseased *Vicia faba* plants and then transferred to a succession of healthy plants. Approximately 100 aphids were transferred to each of the plants tested

Period	Length of exposure	Plants exposed	Plants infected
1st .....	1 hr.	5	2
2nd .....	24 hrs.	5	4
3rd .....	3 days	5	0
4th .....	3 days	5	0
5th .....	3 days	5	0

exposed to aphids immediately after removal from diseased plants became infected. Four of the second set of plants became diseased, but no infection was obtained in any of the succeeding sets of plants. This experiment demonstrated that the pea aphid is able to acquire and transmit vein-mosaic virus of red clover in a total period of 2 hours. It also showed that colonies continue to be infective after feeding periods of 1 hour on healthy plants, but again confirmed the fact that the virus is lost by the aphids while feeding for 1 day on healthy plants. It further indicated that the aphids do not again become infective during a prolonged period on healthy plants.

*Transmission by Other Insects than the Pea Aphid.*—Having found vein-mosaic virus of red clover to be transmitted by the pea aphid, it seemed of interest also to test the potato aphid, *Macrosiphum gei*, and the bean aphid, *Aphis rumicis* L., as possible vectors of the vein-mosaic virus. In several tests, potato aphids were fed on diseased red-clover and *Vicia faba* plants and then transferred or allowed to migrate to healthy *V. faba* or crimson-clover plants. In like manner bean aphids were colonized on diseased *V. faba* plants and then either transferred or allowed to migrate naturally to healthy plants. No infections were obtained in any of the plants.

## DISCUSSION

The experiments presented in this paper demonstrate a method of transmission of vein-mosaic virus of red clover by the pea aphid, resembling that previously shown for the transmission of pea virus 2 by this same vector (3). The pea aphid was found to acquire and transmit the vein-mosaic virus within a total period of 2 hours and to lose the virus when transferred to healthy plants for a period of 1 day. Red clover vein-mosaic virus differs, however, from pea virus 2 in host range and in symptoms produced on diseased plants. The Perfection garden pea, a variety found susceptible to vein-mosaic virus of red clover, is not susceptible to pea virus 2, while several varieties of beans susceptible to pea virus 2, failed to become infected when inoculated with the vein-mosaic virus. Vein-mosaic virus of red clover appears to be distinct from any of the virus diseases previously described in red clover. The mosaic virus of red clover described by Zaumeyer and Wade (5), produces a mottling type of symptoms on *Vicia faba* and peas very different from the symptoms produced by the vein-mosaic virus. The latter is also distinct from 4 viruses described by Pierce (4) and reported to be transmissible to red clover. Black (1) has shown that potato yellow dwarf is transmissible to red clover. Yellow dwarf of potato, however, differs distinctly from the vein-mosaic virus in host range.

The symptoms produced by vein-mosaic of red clover, when transmitted to peas or *Vicia faba*, resemble in some respects those produced by Zaumeyer's pea streak<sup>1</sup> (6), though the evidence available is not sufficient to determine whether or not these two viruses are strains belonging to a single virus group. Pea streak does not produce the whitish bands on the under surface of *V. faba* leaves, a symptom characteristic of the vein-mosaic virus.

Vein-mosaic virus of red clover has been observed in volunteer red clover plants growing in fields and along roadsides. It has not been observed in garden pea plants in gardens where a large percentage of the pea plants were infected with either pea virus 1 or pea virus 2. It was obtained in a single case from a diseased sweet pea plant naturally infected in a garden and also in one case from a *V. faba* plant naturally infected in the field.

## SUMMARY

The mosaic disease caused by a virus designated as the vein-mosaic virus of red clover was transmitted from red clover to *Vicia faba*, sweet pea, red clover, white clover, alsike clover, and crimson clover by means of the pea aphid.

By mechanical methods the virus was transmitted to *Vicia faba*, red clover, white clover, alsike clover, crimson clover, white sweet clover, and several varieties of garden pea.

<sup>1</sup> A culture of pea streak was furnished by Dr. W. J. Zaumeyer for comparison with red clover vein-mosaic virus.

The virus was found to be active after heating to a temperature of 58° C. for 10 minutes, but was inactivated when heated to 60° C. for 10 minutes. It was active after aging *in vitro* for 2 days, but was inactivated after 3 days' aging.

No incubation period of the virus was observed in colonies of the pea aphid that were fed on diseased plants and were then transferred to a succession of healthy plants for a total period of 14 days.

The pea aphid was found to acquire and transmit the virus within a total period of 2 hours. The virus was lost by the aphids during a feeding period of 1 day on healthy plants.

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## BREEDING FOR RESISTANCE TO LATE BLIGHT IN THE POTATO<sup>1</sup>

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### INTRODUCTION

Late blight of potato, caused by the fungus *Phytophthora infestans* (Mont.) de Bary, is so common in the sections of the United States best suited for potato culture and causes such heavy losses that it is often referred to there as "the potato disease."

Severe epidemics frequently occur in the New England States, and less frequently, though all too often, in New York, New Jersey, Pennsylvania, Ohio, Maryland, Michigan, Wisconsin, Minnesota, in the Santa Maria section of California, in the mountain districts of the Southern and Western States, and in Florida on the early crops. In some years and in certain States late blight causes very little damage; in other years (1927, 1928, 1932, and 1936), large losses were sustained by the growers. The largest loss in the last 10 years, according to reports of the Division of Mycology and Disease Survey, Bureau of Plant Industry, occurred in 1928 and amounted to 31,000,000 bushels. During that year late blight was reported from 15 States with the loss in New York alone given as approximately 13,000,000 bushels. In 1932 the crop reduction was estimated as about 9,000,000 bushels, nearly all of which was reported from Maine. The disease reached epidemic proportions in Maine again in 1936. These epidemics have occurred despite the efforts of growers to control the disease by spraying. Since control measures have failed to protect the crop sufficiently in years favorable for the development of late blight, interest in breeding varieties resistant to this disease has greatly increased in recent years.

To find varieties of potatoes more or less resistant to late blight is a simple problem, but to produce varieties with resistance in combination with other characters of economic importance, such as high yield, good market and cooking quality, to say nothing about resistance to the various other diseases, is a more difficult task. It has long been known that some cultivated varieties show various degrees of resistance, but, so far, no variety of this group, *Solanum tuberosum* L., has been found immune from infection by physiologic races of *Phytophthora infestans* prevalent in this country. Varieties have been brought into this country from time to time that were distinctly more resistant than the sorts commonly grown here, but, because

<sup>1</sup> The research here reported was carried on in cooperation with the Maine Agricultural Experiment Station, Orono, Maine.

of their extreme lateness, low yield, poor quality, or other undesirable characters, they never became popular with growers.

Some of these foreign varieties, together with certain seedlings obtained from crosses and selfed lines, have become the foundation stock in the breeding program that has been developed by the U. S. Department of Agriculture in recent years. A report of some of this work has already been published.<sup>2</sup> The present paper gives an account of recent results.

#### MATERIALS AND METHODS

The following resistant varieties have been used as parents in various crosses and selfed lines: No Blight, a variety described by Bonde<sup>3</sup> under the name Foster's Rustproof, is resistant to blight and has been used not only as a parent but as a check in all of the tests for resistance. Ekishirazu, a variety introduced from Japan, is resistant, but produces low yields of malformed tubers. Ackersegen, a yellow-fleshed variety, obtained in Germany, is resistant not only to late blight but to common scab and potato wart, but yields are comparatively low and its tubers are not well shaped. Seedling 45349, a resistant variety from a progeny of Katahdin, naturally pollinated, produces fair yields. These resistant varieties have been crossed with Katahdin, a variety susceptible to the disease, but carrying probably 2 genetic factors for resistance, both in a heterozygous condition. Katahdin is valuable as a parent for other reasons. It produces an almost ideal type of tuber, is resistant in the field to mild mosaic, is vigorous and high yielding and produces an abundance of viable pollen under a wide range of environmental conditions. Seedling 45075 is a highly self-fertile variety, as early as Irish Cobbler, yielding about the same as the latter, but producing a much smoother type of tuber.

Progenies of the blight-resistant varieties crossed with Katahdin are being used with promising results in an effort to get late varieties resistant to the disease. Seedling 45075 is being used as a parent in an attempt to combine earliness and other desirable characters with resistance.

The method employed might be referred to as strain building. This is not a simple or fundamental method in itself but is a system that makes use of a number of breeding methods, namely, introduction, varietal crossing, sib-mating, back-crossing and selfing.

The tests for resistance are carried on in the field at Presque Isle, Maine, and in the greenhouse at Beltsville, Maryland. The seedling varieties are tested in comparison with the variety No Blight, the present standard for resistance, and Green Mountain, which is extremely susceptible to the dis-

<sup>2</sup> Stevenson, F. J., E. S. Schultz, C. F. Clark, W. P. Raleigh, Lillian C. Cash, and R. Bonde. Breeding for resistance to late blight in the potato. *Amer. Potato Jour.* 13: 205-218. 1936.

<sup>3</sup> Bonde, R. A promising blight resistant potato. *Amer. Potato Jour.* 9: 49-54. 1932.

ease. In the experiments conducted in Maine the seedlings are grown in 5-hill lots. Each row of 5-hill lots is grown adjacent to a row of susceptible Green Mountains. As additional checks Green Mountain, No Blight, and the parents of the respective progeny under test are distributed throughout the field.

As soon as the plants begin to show a few blossoms, and when late blight appears in scattered fields along the Aroostook River, but not on Aroostook Farm, infected leaves are collected and used as the source for infection of the blight test plot. The plants are sprayed at intervals during July and August with a water suspension of zoospores, and, if the weather is favorable, a satisfactory epidemic of the disease will result. In 1936, for example, the leaves of susceptible varieties were killed while the tubers were comparatively small, at least 30 days before the plants would have matured normally. In the greenhouse at Beltsville, Maryland, the humidity of the air is raised almost to the saturation point by the addition of steam just prior to the spraying of the plants with the water suspension of zoospores.

#### RESULTS

Two crosses, No Blight  $\times$  Ekishirazu and No Blight  $\times$  Katahdin, together with a number of seedling progenies produced from seed sent from Germany,<sup>4</sup> have been tested for blight resistance for 3 years at Presque Isle in comparison with No Blight, Ekishirazu, Katahdin, and Green Mountain. The data for these tests are given in table 1.

In the 1934 tests the progenies were unselected. The progeny of the cross between two resistant parents, No Blight and Ekishirazu, were found to be in classes 0 to 4. They were all more resistant than the Green Mountain check, with 16.7 per cent of them showing no infection and only 7.3 per cent more heavily infected than either of the parents. It should be noted here that production of tubers by the plants found in class 3 was not noticeably injured by the blight in spite of the fact that lesions were found on many of the leaflets. A plot of class 3 plants is shown in figure 1. It may be seen that no injury is apparent.

Thirty-four of the seedlings of this cross, selected as resistant in 1934, maintained their resistance in the 1935 and 1936 tests, none of them being more heavily infected than class 3, as shown by the table.

The cross between a resistant variety, No Blight, and a susceptible one, Katahdin, showed, as was to be expected, a lower degree of resistance than the cross between the two resistant varieties, No Blight and Ekishirazu. None of the seedlings of the No Blight  $\times$  Katahdin cross were found in the 0 class in 1934, while 2.1 per cent showed a lighter infection than No Blight and 7.6 per cent about the same reaction. In spite of this, the cross as a

<sup>4</sup> The seed was received from K. O. Müller, Berlin-Dahlem, Germany.

TABLE 1.—Reactions of progenies to late blight tested in the field at Presque Isle for 3 years, 1934, 1935, and 1936. The reactions of the parents of two of the progenies and the Green Mountain checks are given for comparison

Cross or variety	Year tested	Seedlings tested	Check plots	Classes of infection <sup>a</sup>						
				0	1	2	3	4	5	6
		No.	No.	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
No Blight × Ekishirazu ...	1934	150		16.7	0	76.0	3.3			
	1935	34		2.9	50.0	29.4	17.6	4.0		
	1936	34			8.8	61.8	29.4			
No Blight × Katahdin .....	1934	340			2.1	7.6	52.1	22.1	14.7	1.5
	1935	31			12.9	25.8	48.4	0	12.9	
	1936	31			3.2	35.5	48.4	12.9		
Progenies of German races .....	1934	340		51.8	10.9	10.9	12.4	7.1	5.6	1.5
	1935	86		26.7	39.5	24.4	8.1	1.2		
	1936	84		84.5	4.8	3.6	7.1			
No Blight .....	1934		21			100				
	1935		50		18.0	64.0	18.0			
	1936		141			45.4	54.6			
Ekishirazu .....	1934		21		90.5	9.5				
	1935		3		100					
	1936		30		30.0	66.7	3.3			
Katahdin .....	1935		24					8.3	91.7	10.3
	1936		29						24.1	65.5
Green Mountain .....	1934		35						100	
	1935		44					22.7	68.2	9.1
	1936		173						31.8	51.4

<sup>a</sup> 0 = Free from late blight.

1 = Occasional leaf with late blight spots.

2 = 8–12 compound leaves per hill with late blight spots on a few of the leaflets.

3 = Approximately half of leaflets with late blight spots.

4 = Approximately  $\frac{2}{3}$  of leaves dead.

5 = All but apical leaves killed, stalks green.

6 = Stalks green, all leaves killed.

7 = All leaves and stalks killed by late blight.

whole showed considerable resistance, since 61.8 per cent were found in classes 1 to 3 and, as stated above, none of these classes was visibly injured with respect to tuber production by the disease. In 1935 and 1936, 31 of the resistant selections of this cross were tested again. In 1935, 4 of these were found to be in class 5, showing as much infection as some of the Green Mountain checks, but in 1936 they were all more resistant than Green Mountain. A relatively large percentage of the seedlings of the progenies of the German races were highly resistant to blight; in 1934, 51.8 per cent escaping infection, and 85.8 per cent being in classes 0 to 3, inclusive. Seedling varie-



FIG. 1. Seedlings of a progeny segregating in the first generation for resistance to late blight. No. 319 (left) practically free from late blight, classed as 3 in the data; No. 320 (center) completely killed by the disease, classed as 7; (right) susceptible Green Mountain check. Photographed on Aug. 21, 1936.

ties resistant in 1934 were resistant again in 1935 and 1936. The pedigrees of the German progenies are not well known to the authors, but they supposedly originated from species hybrids.

In addition to the foregoing, six progenies were tested for 2 years at Presque Isle, Maine, in comparison with their parents and Green Mountain. In this group the progeny showing the greatest resistance was from a cross between 2 resistant parents, S 45349, a seedling of Katahdin, and Ekishirazu. Of the seedlings of this cross 5.6 per cent escaped infection in 1935 and 88.1 per cent were found in classes 0 to 3 inclusive as shown in table 2.

TABLE 2.—*Reactions of progenies to late blight tested in the field at Presque Isle for 2 years, 1935 and 1936. The reactions of the parents and Green Mountain checks are given for comparison*

Cross or variety	Year tested	Seedlings tested	Check plots	Classes of infection <sup>a</sup>							
				0	1	2	3	4	5	6	7
				Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
45349 × Ekishirazu .....	1935	502	No.	5.6	11.2	40.0	31.3	9.2	2.8		
	1935 <sup>b</sup>	166		11.4	13.9	38.0	36.7				
	1936	166		4.2	19.9	46.4	27.7	1.8			
45349 × Katahdin .....	1935	316		0.6	0.9	8.5	18.7	30.4	26.3	14.6	
	1935 <sup>b</sup>	100		2.0	4.0	28.0	66.0				
	1936	100			4.0	30.0	34.0	22.0	9.0	1.0	
Akersegen × Katahdin .....	1935	562		0.5	2.0	8.5	16.9	19.2	29.4	23.5	
	1935 <sup>b</sup>	119		3.4	9.2	35.3	52.1				
	1936	119			5.0	23.5	47.1	22.7	1.7		
No Blight × 45075 .....	1935	425				3.1	9.4	21.4	40.0	26.1	
	1935 <sup>b</sup>	48				18.7	79.2	2.1			
	1936	48				2.1	37.5	37.5	22.9		
Akersegen × 45075 .....	1935	412				2.2	4.9	13.1	34.5	45.4	
	1935 <sup>b</sup>	28				3.6	28.6	64.3	3.6		
	1936	28					7.1	42.9	42.9	7.1	
45349 × 45075 .....	1935	643				1.1	8.7	15.1	25.8	49.3	
	1935 <sup>b</sup>	58				13.8	86.2				
	1936	58			1.7	17.2	34.5	43.1	3.4		
Katahdin selfed .....	1935	241			0.4	0.8	5.0	10.8	49.8	33.2	
	1935		4		25	75					
	1936		17			29.4	70.6				
Ekishirazu .....	1935		3		100						
	1936		30		30.0	66.7	3.3				
	1935		5		40.0	20.0	40.0				
Akersegen .....	1936		22		9.1	90.9					
	1935		50		18.0	64.0	18.0				
	1936		141			45.4	54.6				
Green Mountain .....	1935		44					22.7	68.2	9.1	
	1936		173						31.8	51.4	16.8
	1935		6					8.3	33.3	66.7	100.0
45075 .....	1936		33						91.7	65.5	10.3
	1935		24						24.1		
	1936		29								
Katahdin .....											

<sup>a</sup> See table 1 for legend.<sup>b</sup> The seedlings selected in 1935 and tested again in 1936.

In 1936, 166 seedlings of the latter group were tested again; all but 3 selections were found in the same range of infection as in the preceding year. The progenies of the two crosses, S 45349  $\times$  Katahdin and Ackersegen  $\times$  Katahdin, are quite similar in their reactions to late blight. In each case about 25 per cent of the seedlings were found in the classes 0 to 3 in 1935. The selections made from these classes showed a wider range of infection in 1936 than was to be expected. This was due partly to the fact that the selections were grown as single plants in 1935 and partly to the heavier epidemic of 1936, when they were grown in 5-hill plots. It will be observed that only a small percentage of these selections were as badly infected as the Green Mountain checks.

The 3 crosses between the resistant varieties No Blight, Ackersegen, S 45349, and the susceptible early variety S 45075, show a high degree of susceptibility. None of the seedlings of these 3 crosses escaped the disease and only about 10 per cent of them were found in classes 0 to 3 in 1935. The 1936 test of the selections from these classes showed a still wider range in degrees of susceptibility, for reasons cited above.

It is seen then that the progenies of crosses between two resistant varieties are highly resistant to late blight.

That susceptible varieties differ both phenotypically and genotypically also is evident. From the check plots recorded in table 2, S 45075 is slightly more susceptible to late blight than Katahdin. A comparison between the two crosses No Blight  $\times$  Katahdin and No Blight  $\times$  45075 indicates a similar genotypic difference. In No Blight  $\times$  Katahdin 61.8 per cent of the seedlings were found in classes 1 to 3 as compared with 12.5 per cent for the No Blight  $\times$  45075 cross. Further evidence that Katahdin carries factors for resistance in a heterozygous condition is found in a progeny of Katahdin selfed tested in 1935. Of this progeny 6.2 per cent were in classes 1 to 3 (Table 2). A number of resistant seedlings were found also in a cross between Chippewa and Katahdin, both of which are susceptible. This condition indicates multiple factors cumulative in effect.

#### TUBER RESISTANCE

The discussion thus far has been based entirely on vine resistance, but the tubers of a number of the vine-resistant seedling varieties are resistant also to rot caused by the late-blight fungus. In tests in which the tubers were surface-inoculated with spores of *Phytophthora infestans*, and placed in a moist chamber maintained at a temperature conducive to development of rot, the tubers of some of the resistant seedlings remained sound except where the skin was broken. The Green Mountain check rotted soon and completely (Fig. 2).

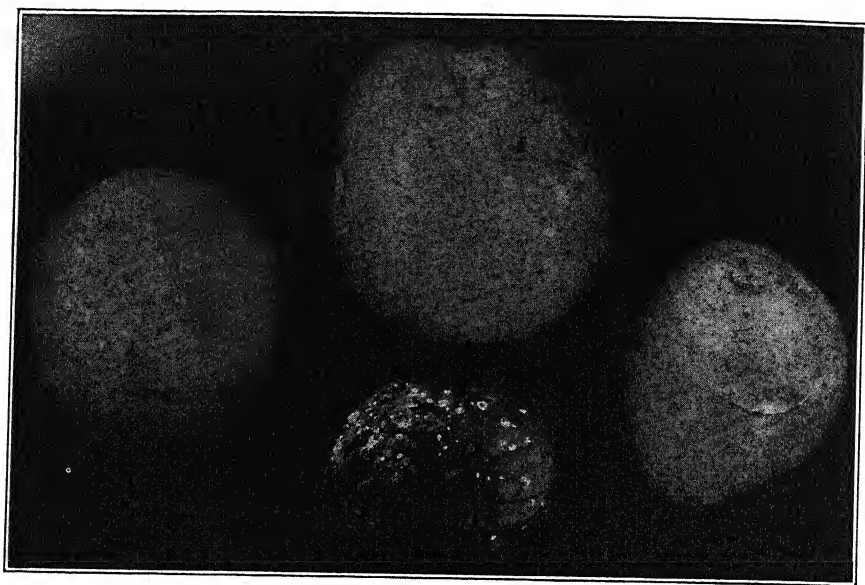


FIG. 2. Resistance of tubers to late blight. Sound tubers from a selection of the No Blight  $\times$  Katahdin cross; decayed tuber from Green Mountain. Both lots surface inoculated with spores of *Phytophthora infestans* in the same moist chamber.

#### COMMERCIAL TYPES

The most promising seedling varieties are not always found in the most resistant progenies. Although all the progeny of the cross No Blight  $\times$  Ekishirazu was highly resistant to late blight, the shapes were so poor and the yields so low that none of the seedlings could be considered as a commercial possibility. The same can be said for the selections from the German progenies; many of them show the highest degree of resistance so far obtained in these experiments, but they are all mediocre in appearance and performance. They will be used in future breeding work, but none will be distributed to growers. The most promising seedling varieties so far produced are No. 44488, a selection from the cross Chippewa  $\times$  Katahdin, several from No Blight  $\times$  Katahdin and a number of others from 45349  $\times$  Ekishirazu. Some of these have been tested several years for resistance, yield, and other characters. The yield data are given in table 3.

Seedling 44488 has outyielded Green Mountain by 18 bushels per acre for a 5-year average. This is not a significant difference in the light of the standard error for these tests, but it does show that a combination of blight resistance and high yield can be secured. The selections from the cross 336 (No Blight  $\times$  Katahdin), have been tested for yield for 2 years. Two of these selections, 336-123 and 336-144, are in the same class as Green Moun-



TABLE 3.—Yields in bushels per acre of U. S. No. 1 tubers of the seedling varieties resistant to late blight in comparison with their parents and standard varieties. Tests made at Presque Isle, Maine

Varieties	Year tested					Average, 1932— 1936	Average 1935— 1936
	1932	1933	1934	1935	1936		
Green Mountain .....	358	233	435	354	518	380	436
Rural New Yorker...	350	183	395	297	474	340	386
Irish Cobbler .....	375	173	446	232	408	327	320
Chippewa .....	392	204	487	310	480	375	395
Katahdin .....	383	195	445	269	432	345	351
No Blight .....	.....	134	447	198	330	277 <sup>c</sup>	264
S44488 <sup>a</sup> .....	433	243	504	317	492	398	405
336-1 .....	.....	.....	.....	300	466	.....	383
336-18 .....	.....	.....	.....	252	515	.....	384
336-83 .....	.....	.....	.....	258	513	.....	386
336-96 .....	.....	.....	.....	275	473	.....	374
336-123 .....	.....	.....	.....	316	534	.....	425
336-144 .....	.....	.....	.....	324	494	.....	409
336-202 .....	.....	.....	.....	163	552	.....	358
336-302 .....	.....	.....	.....	331	412	.....	372

<sup>a</sup> Selection from the cross Chippewa × Katahdin.

<sup>b</sup> The 336 selections are from No Blight × Katahdin.

<sup>c</sup> 4-yr. average with corresponding average for Green Mountains being 385.

tain with respect to yield, if twice the standard error of a difference be taken as the range of experimental error. These same two outyielded the Katahdin parent and all of them significantly outyielded the No Blight parent.

It should be remembered that the yield test plots from which the data in table 3 were secured were all carefully sprayed with Bordeaux mixture. Under these conditions the susceptible varieties are not at a disadvantage. When the plots are grown without spray the results are, however, quite different, as shown in a test made by Reiner Bonde in 1936. In this test two blight-susceptible varieties, Green Mountain and Houma, were grown in a non-sprayed plot in comparison with two resistant ones, No Blight and S 44488 (Table 4).

These varieties were grown in a very wet soil favorable for blight and tuber decay. The foliage of the Green Mountain and Houma varieties was

TABLE 4.—Yields in bushels per acre of susceptible and resistant varieties of potatoes grown in a nonsprayed plot and subjected to a severe late blight epidemic at Presque Isle in 1936

Variety	Type	Yield		Per cent No. 1	Amount of tuber decay
		Total	U.S. No. 1		
Green Mountain	Susceptible	184	140	70	Extensive, 50 per cent or more
Houma .....	"	146	110	70	" " " " " "
No blight .....	Resistant	415	330	73	None noted
S 44488 .....	"	327	308	86	" "

killed early by the late-blight fungus. No Blight and S 44488 were not severely injured, although both developed some infected foliage. The tubers of Green Mountain and Houma decayed badly before being dug, 50 per cent or more of them showing rot at harvest time. In contrast to this, no tuber decay was found on No Blight and S 44488. The two resistant varieties far outyielded the susceptible ones. No Blight outyielded S 44488 significantly in total tubers produced, but the differences between the yields of U.S. No. 1's of these two varieties is not significant when the standard error of the experiment is considered, because only 80 per cent of the tubers produced by No Blight were No. 1, as compared to 94 per cent for S 44488.

The market quality of the U.S. No. 1 tubers produced by S 44488 was far superior to those produced by No Blight. The latter produced a large number of undersized tubers that just made the grade, while S 44488 produced very few small ones, and the general appearance of the U.S. No. 1's was excellent.

The 3 varieties, S 44488, 336-123 and 336-144, produce tubers of desirable shape with shallow eyes. Preliminary cooking tests show that 44488 and several of the selections from the No Blight  $\times$  Katahdin cross rank as high in quality as Green Mountain, when grown on Aroostook Farm at Presque Isle.

#### DISCUSSION

It is evident from the data on the various progenies tested that late-blight resistance in the cultivated potato varieties is inherited as a recessive character controlled by multiple genes. Resistance has been obtained by crossing 2 susceptible sibs and by inbreeding a susceptible variety. Certain progenies of crosses between 2 resistant varieties are all resistant. The progeny of the cross No Blight  $\times$  Katahdin gave a higher percentage of resistant seedlings than did No Blight  $\times$  45075. This indicates that Katahdin is genotypically more resistant than 45075, although phenotypically there is little difference. In contrast to these results a number of reports show that the immunity of *Solanum demissum* is inherited as a dominant character, although there is some reason to believe that the results obtained in *demissum*  $\times$  *tuberosum* crosses may be due to the immunity of the *demissum* in combination with the recessive factors for resistance of the *tuberosum* varieties. This is quite evident from the data of E. Schmidt, a plant breeder of the firm von Kameke, as reported by Bukasov.<sup>5</sup> In this it is shown that the  $F_1$  of *S. demissum*  $\times$  *tuberosum* was 100 per cent resistant, but that the resistance of the subsequent back crosses varied to a considerable degree, depending upon the *tuberosum* variety used. The dominant immunity of *S. demissum* and the recessive resistance of the *tuberosum* varieties are analogous to the genetic

<sup>5</sup> Bukasov, S. M. The problems of potato breeding. Amer. Potato Jour. 13: 235-252. 1936.

behavior of the reaction of certain cereals to disease. As an illustration, Clark and Ausemus<sup>6</sup> first pointed out that in the  $F_1$  and  $F_2$  generations of crosses with Hope, its near immunity from stem rust (*Puccinia graminis tritici* Eriks. and Henn.) was inherited as a dominant character, whereas resistance, as in Kota, was inherited as a recessive character, the dominance in both cases being imperfect or incomplete.

In addition to the information that will be especially helpful in the breeding work of the future, results of economic importance also have been obtained.

Many seedling varieties have been produced within the last few years by hybridization and selection that are resistant enough to late blight to be grown successfully without being sprayed with Bordeaux mixture, even when this disease occurs on other varieties in epidemic proportions. A few of these produce tubers of excellent shape with shallow eyes, and preliminary tests show that they rank high in cooking quality. It is evident then that blight resistance, high yield, desirable tuber shape, shallow eyes and good cooking quality can be combined all in the same variety. Several of these varieties are being given more extensive tests by the Maine Agricultural Experiment Station this year, and if they continue to be superior to the standard varieties they will be increased for general distribution.

#### SUMMARY

Late blight in the potato, caused by the fungus *Phytophthora infestans*, occurs frequently in the sections of the United States best suited for potato culture. Epidemics have continued to occur despite the efforts of growers to control the disease by spraying with Bordeaux mixture. Because of this, interest in breeding varieties resistant to blight has greatly increased in recent years.

Resistant varieties have been brought into this country from time to time, but, because of other undesirable characters, none of them has become popular with growers. Some of these, however, together with other seedling varieties obtained from crosses and selfed lines, have become the foundation stock in a program of breeding for blight resistance.

Certain progenies of crosses between 2 resistant varieties are all resistant. A comparatively large number of seedlings produced from seed obtained from Germany have escaped infection for 3 years.

The most resistant progenies do not always give the most promising seedling varieties when characters such as shape and yield are taken into consideration. None of the highly resistant selections from the German progenies can be considered as commercial possibilities. The same is true of another highly resistant progeny, No Blight  $\times$  Ekishirazu.

<sup>6</sup> Clark, J. A., and E. R. Ausemus. Immunity of Hope Wheat from black stem rust inherited as a dominant character. Jour. Amer. Soc. Agron. 20: 152-159. 1928.

Several desirable seedling varieties from the commercial standpoint have been obtained, however, from a cross between two resistant varieties, S 45349 and Ekishirazu, and from a No Blight  $\times$  Katahdin cross and one has been secured from a cross between the two susceptible varieties, Chipewa and Katahdin.

The tubers of a number of the vine-resistant varieties are resistant also to tuber rot. This is shown in both laboratory and field tests. In plots sprayed with Bordeaux mixture, 3 of the resistant varieties tested were in the same class as Green Mountain in yield. In a nonsprayed plot, however, the differences between susceptible and resistant varieties were highly significant.

Blight resistance in the cultivated varieties is inherited as a recessive character controlled probably by multiple genes.

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## EFFECT OF THE GENETIC CONSTITUTION OF THE HOST ON THE VIRULENCE OF *PHYTOMONAS STEWARTII*<sup>1</sup>

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### INTRODUCTION

It has been shown that virulence of pathogenic cultures of bacteria may be modified by changing environmental conditions. Edwards (3) changed a weakly pathogenic R (rough)-type culture of *Shigella equirulis* into the more virulent S (smooth)-type culture by growing the organisms continuously on nutrient-agar slants of acid reaction at temperatures slightly above 37° C. Koser and Styron (9) were able to convert R-type cultures of *Bacterium dysenteriae* into the S-type by daily or twice-daily transfers in glucose broth. <sup>2</sup> Felton and Dougherty (5) brought about an increase in the virulence of R-type cultures of *Pneumococcus* in milk media by employing a rapid automatic transferring device. The effect of certain chemicals, specific immune sera, physical state of medium, and aging of cultures in bringing about changes in the direction of decreased virulence has been pointed out by Arkwright (2), Hadley (6), Koser and Styron (9), Müller (11) and a score

<sup>1</sup> Journal paper No. J379 of the Iowa Agricultural Experiment Station, Genetics Section, Project No. 404. Part of the work was done at the Rockefeller Institute for Medical Research, Department of Animal and Plant Pathology, Princeton, New Jersey.

<sup>2</sup> The author wishes to express his appreciation to Dr. E. W. Lindstrom for his many helpful suggestions throughout the course of this investigation and for aid in preparation of the manuscript.

of others. More recently, Ark (1) reported that 10 per cent sucrose broth decreased the invasive capacity of cultures of *Erwinia amylovora*, while 2 per cent sucrose broth seemed to restore the virulence. These citations will suffice to show that changes away from or towards pathogenicity may be brought about *in vitro*.

There are a few reports to show that virulence may also be changed *in vivo* by passage through plants or animals. It is commonly believed that bacteria become more virulent by continuous passage through susceptible plants or animals. The effect of continuous culture in *in vivo* environments, such as those furnished by resistant and susceptible hosts, has, however, never been determined. Both Sharp (12) and Elcock (4) reported that S-type variants appeared in R-type cultures of *Phytomonas beticola* and *Phytomonas phaseoli sojense*, respectively, during passage through susceptible plants. Todd (13) has shown that virulence of certain strains of haemolytic streptococci may be enhanced by successive passages through mice, but he does not state whether passages were made through resistant or susceptible mice.

The purpose of this investigation was to study the effect of successive passages through resistant and susceptible hosts on the virulence of *Phytomonas stewarti* (E.F.S.) Bergey *et al.* [*Bacterium stewarti* (E.F.S.)]. This organism causes a disease known as bacterial wilt of maize, also commonly known as Stewart's disease. Beginning with strains of a known degree of virulence, passages were successively made through a very stable, highly resistant, inbred line of maize and an equally stable, highly susceptible, inbred line. Later in the investigation, the effect of hosts other than maize also was studied. Very striking and consistent differences were evident between cultures successively passed through resistant hosts and those passed through susceptible hosts.

#### MATERIAL

##### Host Material

The host material for this investigation consisted chiefly of 2 highly stable inbred lines of maize. One, a yellow-dent inbred (OSF) was highly resistant to *Phytomonas stewarti*; the other, a yellow, sweet Golden Bantam line (GB797), was very susceptible. A very susceptible variety of teosinte and a series of grasses that will be enumerated later also were used. The high genetic stability of the 2 inbred lines and their specific reactions to *Phyt. stewarti* has been described in an earlier paper (14) in connection with studies on the mode of inheritance of resistance to bacterial wilt in maize. In brief, the inbred GB797 was very susceptible, usually dying within a period of 14-20 days after inoculation with a virulent strain of the organism. OSF, when inoculated with the same strain, showed characteristic lesions shortly after inoculation, but readily recovered. Usually no signs of the

disease, with the exception of a few dried-up lesions, were apparent on OSF after 3-4 weeks. The reaction of these 2 host lines is definitely inherited on a genetic basis.<sup>3</sup> At least 3 dominant supplementary factors are involved in the production of high resistance. The absence or recessive condition of the 3 factors results in complete susceptibility. OSF was homozygous for all 3 dominant factors, whereas GB797 was homozygous for the triple recessive condition.

### Cultures Used

The initial strains of *Phytophthora stewarti* used in these studies were designated S15 and FB32. S15 was a weakly virulent, single-colony isolation from a weakly virulent culture that had been grown on nutrient-dextrose agar for more than 2½ years. FB32 was a highly virulent single-colony isolation from the highly virulent culture used in studies on the mode of inheritance of resistance to bacterial wilt in maize.<sup>3</sup> The weakly virulent strain S15 produced relatively few water-soaked lesions on seedlings of the susceptible inbred line GB797, and, although it severely stunted the plants, it was unable to kill them. In comparison, the highly virulent strain FB32 produced many prominent lesions on seedlings of this host and killed the plants usually within a period of 14-20 days after inoculation. Both strains had a very mild effect on seedlings of the resistant inbred OSF. Lesions produced by FB32 on this host were more numerous and prominent than those produced by S15, but, in either case, OSF recovered without a noticeable degree of stunting.

The 2 strains differed in type of growth on nutrient-dextrose agar, as well as in virulence. The weakly virulent strain S15 showed a very firm, rather dry type of growth on nutrient-dextrose-agar slants. When it was plated out the colonies invariably were small, bead-like and very firm. The highly virulent strain FB32 showed a slimy or watery type of growth on nutrient-dextrose-agar slants. Considerable liquid or slime usually collected at the bottom of the slant. When FB32 was plated out the resulting colonies invariably were large, flat, and watery and had a tendency to spread over the media.

### METHODS

Two subcultures of each of the 2 strains S15 and FB32 were made. The 2 subcultures of S15 were designated S15-O and S15-B. Similarly the 2 subcultures of FB32 were designated FB32-O and FB32-B. S15-O and FB32-O were successively passed through the resistant host OSF. S15-B and FB32-B were successively passed through the susceptible host GB797. Numbers following O (from OSF) or B (from the Bantam inbred GB797)

<sup>3</sup> Wellhausen, E. J. Genetics of resistance to bacterial wilt in maize. 1936. [Unpublished doctorate thesis. Copy on file, Library, Iowa State Col., Ames.]

were used to designate the number of passages; for example, S15-O after the first passage through OSF was designated S15-O1, after the second passage, S15-O2, and so on.

The 4 cultures S15-O, S15-B, FB32-O, and FB32-B were handled exactly alike, *i.e.*, all 4 were put into the respective resistant or susceptible plants on the same day or hour and reisolated at the same time at a later date. The organisms were injected into the growing point of week-old plants by means of a standard hypodermic syringe. Fifteen plants per culture were used. The time interval between inoculation and reisolation in the first 3 passages was 7-10 days and thereafter 14-16 days. Two plants per culture from the 15 inoculated were taken for reisolation. Upon reisolation, 10-15 colonies per culture were picked from the plates at random and transferred to agar slants. The progeny of these 10-15 colonies pooled constituted the inoculum for the next passage. The object of picking a number of colonies per culture was to obtain a more representative sample of the population for the next injection. If the plants were not quite ready when the cultures were ready for the next passage the organisms were stored in a refrigerator at 10° C. The organisms might have been transferred directly from plant to plant by means of infected tissue, but the method employed offered an opportunity to study colony types.

Later in the investigation FB32-O, after 14 passages through the resistant maize host (OSF), was transferred to teosinte, sorghum, millet, and other grasses and successively passed through them, according to the above methods.

Virulence of the various strains after a number of host passages was tested usually on the susceptible inbred GB797. Strains to be compared were tested under the same conditions. Injections were made into the growing point of 4 to 7-day-old seedlings grown in 16 × 22 × 3 inch flats, 5 rows per flat, 15 plants per row. The degree of virulence was determined chiefly on the basis of time required for first lesions to appear, the number of lesions present on a unit number of plants 7 days after inoculation, rate of wilting and the degree of stunting in comparison with the noninoculated controls after a certain period of time, usually 2 weeks.

In determining the number of lesions (water-soaked streaks along the veins of the leaf) produced by a certain strain of the organism, one is confronted with many variables. The lesions or streaks are not uniform in size or type. Often many of them coalesce making a broad irregular stripe, and very frequently if the organisms are highly virulent many of the leaves are killed in 3 or 4 days. In order to take some of these variables into consideration an arbitrary method of counting was devised. Generally 15 or 24 plants were used as a unit. Distinct narrow streaks were each counted as 1 lesion disregarding minor variations in size or type. Wider streaks were

valued at 2 or more lesions, depending on the width. If the entire leaf was dead or in a badly water-soaked condition, it was arbitrarily valued at a maximum of 10 lesions. The total number of lesions obtained in this way divided by the total number of leaves per unit of plants constitutes the lesion index for the various strains of the organism. The chief value of the lesion index is to show the comparative invasive capacity of the different strains or their rapidity of spread within the host. It may be seen later that comparisons based on lesion index are highly correlated with those based on degree of stunting.

In one test the degree of stunting was determined by direct measurements of height of the plants. In the others it was determined on the basis of percentage reduction in green weight 2 weeks after inoculation. Green weight was used in preference to dry weight because certain apparent differences could be ascertained by green weights that were obliterated on a dry-weight basis. Very often, plants inoculated with one strain made the same amount of growth as plants inoculated with another strain, yet the one group may be completely dried up 2 weeks after inoculation, whereas the other may still bear turgid leaves. Such differences may be shown by green but not by dry weights. Green weight of the plants in these experiments includes only the tops, separated from the roots of each plant at the node with permanent root system.

#### EXPERIMENTAL RESULTS

##### Effect of Successive Passages Through OSF (Res.) and GB797 (Susc.) on the Virulence of S15 and FB32

The subcultures of S15, namely S15-O and S15-B, were passed 10 times through seedlings of the resistant host "O" (OSF) and susceptible host "B" (GB797), respectively. Subcultures of FB32, namely FB32-O and FB32-B, were passed 14 times through seedlings of hosts O and B, respectively. Tests for virulence in both series were made after 2, 4, 6, 9, and 10 passages, and in the FB32 series also after the 14th. The first 3 tests were of a preliminary nature, and only general notes were taken. In these S15-O was compared to S15-B and FB32-O to FB32-B after 2, 4 and 6 passages respectively. Comparisons with the original strains were not made until after completion of 9 passages.

In the test after 2 passages, no difference in virulence between the resulting strains S15-O2 and -B2 or FB32-O2 and -B2 was evident. In the test after 4 passages, still no difference between resulting strains of the S15 series (S15-O4 and -B4) could be detected. But in the FB32 series, FB32-O4 was distinctly more virulent than FB32-B4. Eighty-six per cent of the 56 seedling test plants (GB797) inoculated with FB32-O4 were dead<sup>4</sup> 10 days

<sup>4</sup> Plants were considered dead when all the leaves had completely dried up or wilted beyond recovery.



after inoculation. In comparison, only 43 per cent of the 53 inoculated with FB32-B4 were dead at the time. Ten days later, when the plants were discarded, all of those inoculated with FB32-O4 were dead, whereas 33 per cent of those inoculated with FB32-B4 still contained a number of green and turgid leaves.

In the test after 6 passages, a slight difference in the S15 series was first noted. Judging from differences in general appearance and degree of stunting of the test plants, S15-O6 was slightly more virulent than S15-B6. In the FB32 series, the spread in virulence between the resulting strains was very striking. All of the 26 test plants inoculated with FB32-O6 were dead 10 days after inoculation, but none of the 23 inoculated with FB32-B6 was dead at the time. Many of the leaves, however, were severely streaked with water-soaked lesions. When the test was discarded 10 days later, the plants inoculated with FB32-B6 showed signs of recovery. New leaves were being unfolded on which relatively few lesions could be found.

In the test after 9 passages, the virulence of the resulting strains S15-O9 and -B9 and FB32-O9 and -B9 was tested along with the original strains S15 and FB32. In this test more detailed notes and comparative measurements were made. Each culture was injected into 30 4-day-old plants of GB797. The time required for first lesions to appear and the mean plant height (alive or dead) 20 days after inoculation for each series of plants are given in table 1. The actual appearance of the test plants before they were discarded is shown in figure 1. It is clearly evident in either table 1 or figure 1 that the virulence of both S15 and FB32 was increased by successive

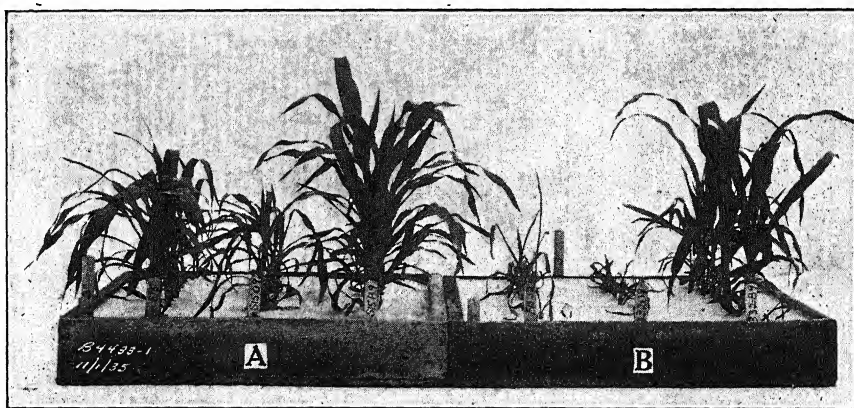


FIG. 1. Comparative effect of cultures S15 and FB32 on the susceptible host GB797 before and after host passage. A. Rows from left to right: 1, weakly virulent culture S15; 2, S15 after 9 passages through OSF (resis.); 3, S15 after 9 passages through GB797 (susc.). B. Rows from left to right: 1, highly virulent culture FB32; 2, FB32 after 9 passages through OSF; 3, FB32 after 9 passages through GB797. Photographed 20 days after inoculation.

TABLE 1.—*Virulence of cultures S15 and FB32 on GB797 before and after 9 successive passages through OSF (resis.) and GB797 (sus.) based on time required for first lesions to appear and mean height of plants 20 days after inoculation*

	S15 series			FB32 series		
	Original culture S15	9 passages through OSF (resis.) S15-O9	9 passages through GB797 (sus.) S15-B9	Original culture FB32	9 passages through OSF (resis.) FB32-O9	9 passages through GB797 (sus.) FB32-B9
Hours required for first prominent lesions to appear .....	50	40	60	40	24	60
Mean height (cms.) of plants 20 days after inoculation (alive or dead) .....	17.0 ± .25 <sup>a</sup>	11.1 ± .38	20.0 ± .52	8.6 ± .13 (dead) <sup>b</sup>	4.4 ± .11 (dead) <sup>c</sup>	18.2 ± .29

<sup>a</sup> Standard error.

<sup>b</sup> Required 20 days to kill all the plants.

<sup>c</sup> Required 7 days to kill all the plants.

passages through the resistant host (OSF) and decreased by successive passages through the susceptible host (GB797).

The increase or decrease in virulence in comparison to the original culture is most striking in the FB32 series. Plants inoculated with the original strain FB32 first showed water-soaked lesions along the veins of the leaf about 40 hours after inoculation. The lesions slowly became more prominent and numerous as the organisms spread throughout the vascular system. The plants wilted gradually, leaf by leaf, but were not completely wilted or dead until 3 weeks after inoculation. The strain, FB32-O9, (FB32 after 9 passages through the resistant host OSF) was considerably higher in virulence. Prominent water-soaked lesions appeared 24 hours after inoculation and all plants were dead 6 days later. They made almost no growth after inoculation. In contrast, FB32-B9 (FB32 after 9 passages through the susceptible host GB797) was much lower in virulence than the parent culture. Water-soaked lesions did not appear until 60 hours after inoculation. The organisms did not spread rapidly. None of the plants died. After 20 days relatively few prominent water-soaked lesions could be detected on the newer leaves of the plants.

In the S15 series similar trends were evident (Table 1 and Fig. 1), but differences were less outstanding. The greatest change in this series seemed to have occurred in the direction of increased virulence. With 9 successive passages through the resistant host the virulence of S15 was brought up approximately to that of FB32. By 9 successive passages through the susceptible host, however, its already quite low virulence was only slightly further reduced. It is of interest to note that S15-B9 and FB32-B9 were very similar in degree of virulence, although the original cultures were very different.

The S15 and FB32 series were tested again along with the original strains after the 10th passages. In this test comparisons were made on both hosts, the susceptible GB797 and the resistant OSF. Differences on GB797 were essentially the same as those described for the 9th passages. On OSF differences between the weakly virulent strains S15, S15-B10 and FB32-B10 were hard to detect. Each produced a few lesions, but, otherwise, had no outward effect. Strains S15-O10 and FB32 produced fairly prominent lesions and a slight degree of stunting. The greatest effect was produced by FB32-O10. Lesions were very prominent and plants severely stunted, as will be shown later, but none was killed.

No further passages were made in the S15 series after the 10th. But in the FB32 series 4 more passages were made to determine whether virulence may be further increased by continued passages through the resistant host or further decreased by continued passages through the susceptible host. Virulence of the 2 strains after the 14th passage was compared with that of

TABLE 2.—Effect of FB32-O14 and -B14 on hosts OSF and GB797 compared on the basis of lesion index and percentage reduction in green weight

Strain	Host	No. of plants	Lesion index	Green weight in grams	Reduction in green weight in grams	Percentage reduction in green weight
Control	OSF	24	0	98.0	0	0
Control	GB797	24	0	69.0	0	0
A. Effect of FB32-O14 and -B14 compared with that of -O10 and -B10 on GB797						
FB32-O14	GB797	24	6.3	2.5	66.5	96.3
-O10	"	24	5.9	4.0	65.0	94.3
FB32-B14	"	24	2.3	21.6	47.4	68.7
-B10	"	24	2.5	24.0	45.0	65.3
B. Effect of FB32-O14 and -B14 on hosts OSF and GB797, respectively						
FB32-O14	OSF	24	1.9	31.2	66.8	68.1
FB32-B14	GB797	24	2.3	21.6	47.4	68.7
C. Effect of cross inoculation						
FB32-O14	GB797	24	6.3	2.5	66.5	96.3
FB32-B14	OSF	24	0.4	72.0	26.0	26.5

the same strains after the 10th. The results in table 2, A, show that practically no further change in virulence was brought about by 4 more passages through the respective hosts. Apparently, a point of stability was reached in both hosts sometime within the first 9-10 passages, beyond which further passages had little effect. Proper tests to determine the exact number of passages required before this point of stability was reached in either of the hosts were not made. However, in view of the preliminary tests it appears that the increase or decrease in virulence was brought about more or less gradually and that a point of apparent stability probably was not reached before 6-7 passages.

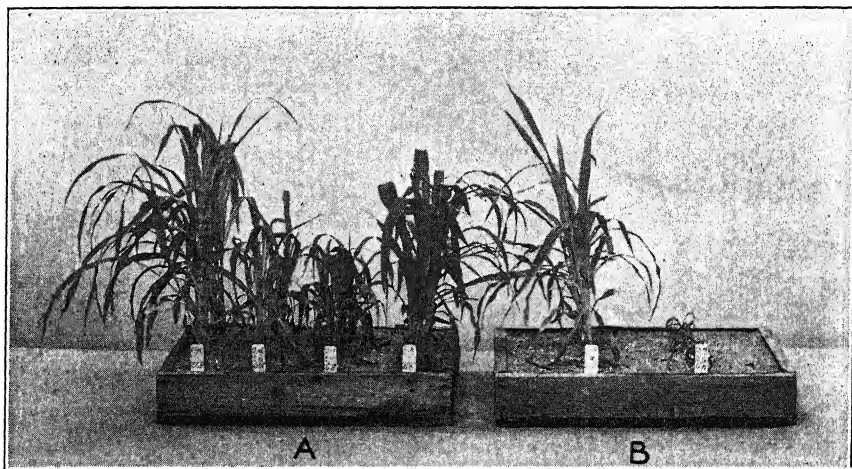


FIG. 2. A. Effect of FB32-O12 on OSF compared with the effect of FB32-B12 on GB797. Rows from left to right: 1, OSF uninoculated control; 2, OSF inoculated with FB32-O12; 3, GB797 inoculated with FB32-B12; 4, GB797 uninoculated control. B. Effect of cross inoculation. Row on left, OSF inoculated with FB32-B12. Row on right, GB797 inoculated with FB32-O12. Photographed 15 days after inoculation.

It may be seen in figure 2, A, and table 2, B, that after the strains had stabilized, the one successively passed through the resistant host OSF had about the same effect on OSF as the strain successively passed through the susceptible host GB797 had on GB797. Both strains, in regard to virulence, seemed to have reached a balance or equilibrium with the resistance of their respective hosts at a point which resulted in a marked degree of stunting but no death of the plants. The effect of cross inoculation (Fig. 2, B, and Table 2, C) shows that the strain in balance with OSF was extremely virulent on GB797, but the strain in balance with GB797 had little effect on OSF.

In order to test whether FB32-O14 and FB32-B14 had changed in specificity in comparison with the original strain FB32, inbred lines, the relative resistance or susceptibility of which had been previously determined

with FB32, were tested with FB32-O14 and -B14. On each of the lines FB32-O14 was found to be more virulent, and FB32-B14 less virulent than the original culture FB32. No changes in regard to relative resistance or susceptibility were revealed; *i.e.*, an inbred line, more resistant than another when tested with FB32, showed the same relationship when tested with FB32-O14 or -B14. The respective increase or decrease in virulence brought about by passage through the particular hosts, therefore, seems to apply to inbred lines of maize in general.

#### Effect of Passages Through Older Plants of OSF on the Virulence of FB32-O14

Possibly FB32-O14 could have been further increased in virulence by passages through a line of maize more resistant than OSF, but such a line was not available. Since older plants were more resistant than seedlings, it was thought that virulence might be further enhanced by passages through older plants of OSF. To determine this, FB32-O14 was successively passed through plants of OSF, 2-3 weeks older than in previous experiments. After 9 passages the virulence of the resulting culture, OSF-23, was compared with that of FB32-O14 on 4-day-old seedlings of both GB797 and OSF. The results (Table 3) show that OSF-23 was slightly higher in virulence than

TABLE 3.—*Virulence of FB32-O14 compared with OSF-23 on hosts GB797 and OSF*

Strain	Host	Lesion index <sup>a</sup>	Percentage reduction in green wt. <sup>a</sup>	Mean difference in lesions	Mean difference in green weight reduction
FB32-O14 .....	GB797	7.4 ± .32 <sup>b</sup>	96.9 ± .25	1.3 ± .42	1.2 ± .26
OSF-23 .....	GB797	8.7 ± .28	98.1 ± .10		
FB32-O14 .....	OSF	.87 ± .03	53.7 ± 6.0	0.33 ± .3	10.7 ± 9.2
OSF-23 .....	OSF	1.2 ± .3	64.4 ± 7.0		

<sup>a</sup> Average of 3 tests (15 plants each).

<sup>b</sup> Standard error of the mean.

FB32-O14, especially on GB797. It is dangerous, however, to conclude that the small difference between the 2 cultures was entirely due to passages through the older plants. It may well be that part or all of it was due to a slight loss in virulence of FB32-O14 during culture on nutrient-dextrose agar while the above 9 passages were being made.

#### Reversion of Virulence in Cultures FB32-O14 and FB32-B14 by Reversing Hosts

In order to determine whether the virulence of the passage strains may be changed by reversing hosts, the highly virulent strain (FB32-O14) was

passed through the susceptible host GB797 and the weakly virulent strain (FB32-B14) was passed through the resistant host OSF. Tests for virulence were first made after 6 passages. The results in table 4 show that FB32-O14,

TABLE 4.—Results of tests for virulence of FB32-O14 after passages through the susceptible host and FB32-B14 after passages through the resistant host

Strain	Host	No. of plants	Lesion index	Green wt. (grams)	Reduction in green weight (grams)	Percentage reduction in green wt.
FB32-O14	GB797	24	6.3	2.5	66.5	96.3
(Control)		24	1.9	31.2	66.8	68.1
FB32-O14	GB797	24	3.0	18.0	51.0	74.0
(After 6 passages through susc. host)		24	.54	65.2	32.8	33.5
FB32-B14	GB797	24	2.3	21.6	47.4	68.7
(Control)		24	.48	72.0	26.0	26.5
FB32-B14	GB797	24	2.7	20.6	48.4	70.1
(After 6 passages through resist. host)		24	.75	74.4	23.6	24.1
FB32-B14	GB797	15	2.8	34.0	63.5	65.2
(After 13 passages through resist. host)		15	.51	66.0	25.0	27.5
OB-6 <sup>a</sup>	GB797	15	3.1	9.0	88.5	91.0
	OSF	15	.94	37.0	54.0	58.5

<sup>a</sup> A second series of 6 passages through the resistant host starting with FB32-B14 a second time.

after 6 passages through the susceptible host, had decreased in virulence. It was almost reduced to the virulence of FB32-B14. FB32-B14, however, after 6 passages through the resistant host, was not noticeably changed. After 13 passages it still had not changed. This particular culture seemed to be highly stable. When no changes were evident after the 6th passage, another series of passages was started from FB32-B14, which, meanwhile, had been maintained on nutrient-dextrose-agar slants in a refrigerator. Tests for increased virulence in this second series were also made after the sixth passage. The culture was labeled OB-6. Table 4 shows the virulence was greatly enhanced in the second series of passages.

#### Decrease in Virulence of FB32-O14 by Passage through Susceptible Teosinte, *Euchlaena mexicana*

The variety of teosinte used in this experiment was almost as susceptible to *Phytophthora stewarti* as GB797. FB32-O14 was successively passed through this host 8 times. Virulence of the resulting culture (Teo-8) was compared with that of FB32-O14 on both the susceptible variety of teosinte and the susceptible line of maize GB797. Teo-8 was found lower in viru-

lence on either host. Seedlings of the susceptible variety of teosinte inoculated with FB32-014 were completely dried up 6 days after inoculation, whereas 75 per cent of those inoculated with Teo-8 were beginning to recover at that time. Teo-8 on GB797, produced fewer lesions and was 20 per cent below that of FB32-014 in green-weight reduction. It is obvious, therefore, that successive passages through the susceptible variety of teosinte had an effect similar to successive passages through the susceptible maize host.

#### Effect of Other Hosts on the Virulence of FB32-014

Ivanoff (8) has shown that *Phytomonas stewarti* will infect sorghum, *Holcus sorghum* L.; Sudan grass, *Holcus sudanensis*; yellow foxtail, *Setaria glauca*; German foxtail millet, *Setaria italica* var. *stramineofructa*; and common millet, *Panicum miliaceum*. The leaf lesions induced by Stewart's wilt organism on these species, with a few exceptions, resembled in shape and manner the development of those induced by the same pathogen on maize. Ivanoff also reported cross inoculations made by using the organism reisolated from sorghum upon maize, Sudan grass, and the millets, and *vice versa*. No change in pathogenicity of the bacteria was observed as a result of the passage through other plants. He does not state whether more than one passage was made.

In investigation here reported, strain FB32-014 (highly virulent on maize) was successively passed through the following grasses<sup>5</sup> in the same manner as described for maize [Scientific names according to Hitchcock (7)]:

- A. Sudan grass, *Sorghum vulgare* var. *sudanense* (Piper) Hitch.
- B. Black amber sweet sorghum, *Sorghum vulgare* Pers.
- C. White kafir grain sorghum, *Sorghum vulgare* Pers.
- D. Grohoma forage sorghum, *Sorghum vulgare* Pers.
- E. Pearl millet, *Pennisetum glaucum* (L.) R. Br.
- F. Proso or broom corn millet, *Panicum mileaceum* L.
- G. Japanese millet, *Echinochloa crusgalli* var. *frumentacea* (Roxb.) Wight.
- H. Foxtail millet, *Setaria italica* (L.) Beauv.
- I. German millet, *Setaria italica* (L.) Beauv. var. *stramineofructa* Bailey.
- J. Smooth brome grass, *Bromus inermis* Leyss.
- K. Rye grass, *Lolium perenne* L.
- L. Reed canary grass, *Phalaris arundenacea* L.
- M. Timothy, *Phleum pratense* L.
- O. Tall meadow oat grass, *Arrhenatherum elatius* (L.) Mert. and Koch.

Leaf lesions developed on all these grasses after inoculation with FB32-014. They were more prominent on Sudan grass, the sorghums, and proso millet than on the others, but in general, each grass was more resistant to

<sup>5</sup> Grasses obtained from the Agronomy Department, Iowa State College.



FB32-O14 than the resistant line of maize, OSF. After FB32-O14 had been passed through each of the above grasses 6 or more times with the exception of brome and rye grass, the resulting strains were tested for virulence. (It was very difficult to reisolate from either brome or rye grass and the passages were discontinued after the first two.) The virulence of each grass-passage strain was compared to that of the original strain, both on the respective grass through which successive passages had been made and on the susceptible maize inbred GB797.

Comparisons on the grasses were made on the basis of average number of lesions per leaf from a total of 25 plants. The results are presented graphically in figure 3. The black bars show the number of lesions produced by

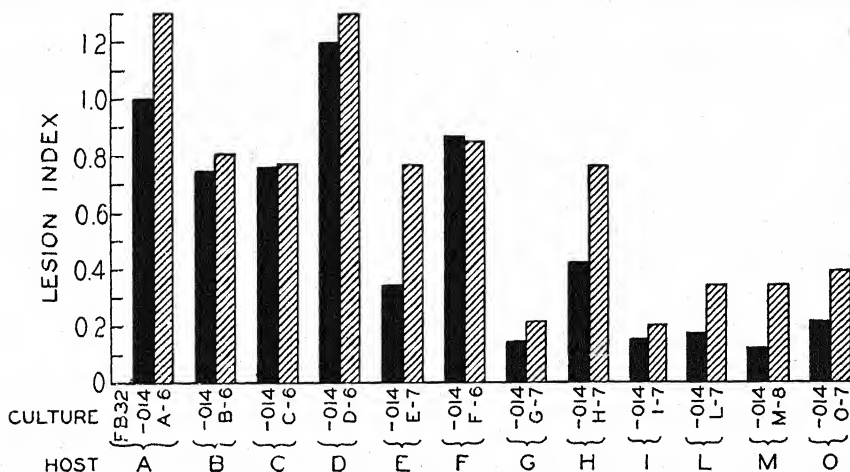


FIG. 3. Effect of FB32-O14 on different grasses before and after 6-8 passages through them. Solid bar shows the effect of FB32-O14 on a particular grass before passage. Shaded bar shows the effect of FB32-O14 on the same grass after successive passages through it. (Passage strains were named according to host and number of passage made; e.g., A-6 = FB32-O14 after 6 passages through host A. See text for hosts corresponding to A, B, C, etc.)

FB32-O14 on each of the grasses listed above with the exception of brome and rye grass. The shaded bars show the lesions produced on the same grasses by FB32-O14 after it had been passed through each grass 6 or more times. The number of passages through each is indicated by numbers following A, B or C, e.g., FB32-O14 after 6 passages through Sudan grass (A) was labeled A-6; FB32-O14 after 7 passages through Reed canary grass (L) was labeled L-7.

It is clearly evident from figure 3 that the majority of the passage strains were more virulent for their respective grass hosts than the original strain FB32-O14. Increases in virulence for their respective hosts were very noticeable in strains A-6, E-7, H-7, L-7, M-8 and O-7. These strains, in addition to producing more lesions, also caused a greater degree of stunting than the original strain (Fig. 4).



FIG. 4. Left, effect of FB32-O14 on pearl millet. Right, effect of FB32-O14 on same host after 7 passages through it. Photographed 14 days after inoculation.

Virulence of the various grass-passages strains for the susceptible inbred line of maize GB797 in comparison with the original strain (FB32-O14) is shown in table 5. Comparisons were made on the basis of lesions 7 days

TABLE 5.—Virulence of grass-passages strains compared with the original strain FB32-O14 on GB797

Strain	Lesion index <sup>a</sup>	Percentage reduction in green wt. <sup>a</sup>	Differences in lesion indexes compared to FB32-O14	Differences in percentage reduction of green wts. compared to FB32-O14
FB32-O14 .....	7.4 ± .32 <sup>b</sup>	96.9 ± 0.25		
A-6 .....	3.9 ± .42	83.3 ± 1.60	3.5 ± .52 <sup>c</sup>	8.6 ± 1.60
B-6 .....	3.7 ± .40	92.3 ± 0.50	3.7 ± .50	4.6 ± 0.55
C-6 .....	6.3 ± .35	93.6 ± 2.00	1.1 ± .46	3.3 ± 2.00
D-6 .....	4.7 ± .56	92.0 ± 1.90	2.7 ± .64	4.9 ± 1.90
E-7 .....	3.4 ± .28	74.6 ± 3.10	4.0 ± .42	22.3 ± 3.10
F-6 .....	4.0 ± .14	95.1 ± 3.60	3.4 ± .34	1.8 ± 3.60
G-7 .....	3.4 ± .35	84.5 ± 4.20	4.0 ± .46	12.4 ± 4.20
H-7 .....	2.0 ± .21	77.6 ± 3.60	5.4 ± .38	19.3 ± 3.60
I-7 .....	5.9 ± .42	94.4 ± 1.80	1.5 ± .52	2.5 ± 1.80
L-7 .....	0.2 ± .04	43.6 ± 4.00	7.2 ± .31	53.3 ± 4.00
M-8 .....	8.7 ± .63	97.8 ± 0.28	+ 1.3 ± .70	+ 0.9 ± 0.30
O-7 .....	0.5 ± .04	57.1 ± 4.40	6.8 ± .31	39.8 ± 4.40

<sup>a</sup> Average of 3 replications, 15 plants per replication.

<sup>b</sup> Standard error of the mean.

<sup>c</sup> Standard error of the mean difference.

after inoculation and percentage reduction in green weight 14 days after inoculation. It may readily be seen in table 5 that, with one exception (M-8), the grass-passage strains that were more virulent for their particular grass hosts were less virulent for GB797. Those showing no particular change in virulence on the grass host also showed comparatively little change on GB797. The greatest reduction in virulence for maize was brought about by passages through Reed canary grass. Effect of the resulting strain, L-7, on Reed canary grass and GB797 in comparison with FB32-014 on the same hosts is shown in figure 5.

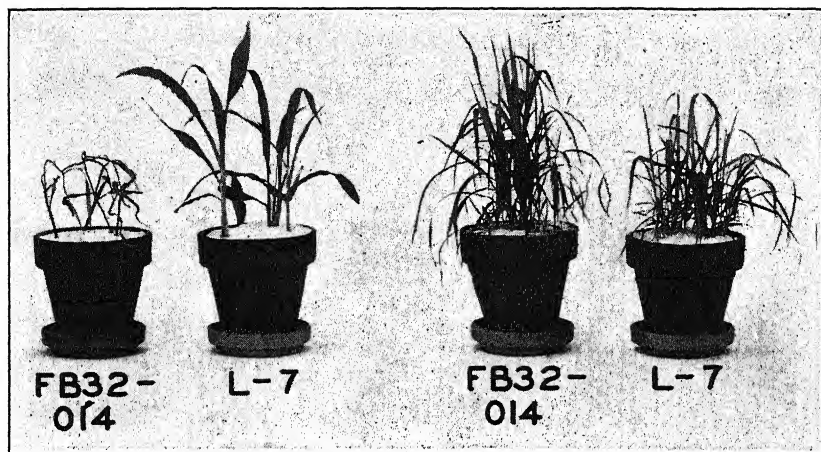


FIG. 5. Effect of FB32-014 and L-7 on GB797 and Reed canary grass, respectively. Two pots on left, GB797. Two pots on right, Reed canary grass. L-7 = FB32-014 after 7 passages through Reed canary grass. Photographed 14 days after inoculation.

It was hoped that a grass might be found that would increase the virulence for maize beyond that of the resistant inbred line OSF. There is some indication of this in the case of timothy. The strain M-8 (8 passages through timothy) was considerably more virulent on timothy and also slightly more virulent on GB797 than was FB32-014. The results, however, should be repeated before any definite conclusions are drawn.

#### General Differences in Culture Characteristics of the Host-passage Strains

Although no detailed cultural studies were made on the various host-passage strains, certain differences in type or manner of growth on nutrient-dextrose agar were noted. Strains, highly virulent for maize, such as FB32-014, OSF-23, I-7 and M-8, were extremely watery or liquid in type of growth (Fig. 6, A), whereas strains, weakly virulent for maize, such as FB32-B14 or S15, tended to be firmer (Fig. 6, B). As FB32-014 was re-

duced in virulence by passages through the susceptible host GB797 it also became firmer in growth. Likewise, when FB32-B14 was increased in virulence by passages through the resistant host OSF, it became more watery or slimy. Strains, intermediate between the extreme types of virulence, also had a tendency to be intermediate in degree of liquidity or sliminess.

Colonies of the weakly virulent strains usually were small and firm. Those of S15 were inclined to be rough. Colonies of the highly virulent strains were large and had a tendency to spread over the media. When the Petri dishes were tilted the colonies tended to run down the slant.

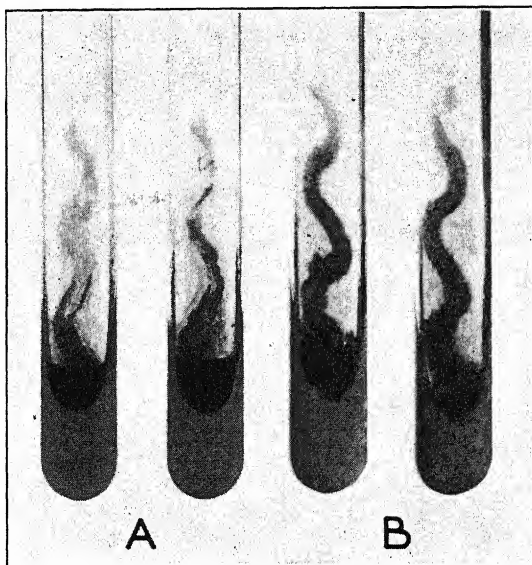


FIG. 6. Differences in type of growth on nutrient-dextrose-agar slants of a highly virulent strain (A) and a weakly virulent strain (B). Note collection of liquid at bottom of slant in both tubes of A.

L-7, the strain obtained from FB32-O14 by 7 successive passages through Reed canary grass, was the only strain in which changes other than those described above were noted. This strain, as it gained virulence for Reed canary grass by successive passages through it, lost certain type characteristics of *Phytomonas stewarti* with respect to color and manner of growth on nutrient-dextrose agar, as well as its virulence for maize.

#### DISCUSSION

At this stage of the investigation it is impossible to give a final interpretation of the methods whereby the above changes were brought about. It may be that the host environment has a direct effect on the organism, bringing

about temporary changes that may last through several generations. On the other hand, the changes in the various strains may have come about through random mutation and natural selections of those organisms best adapted to the particular environment of the host. There is reason to expect that the same biological phenomena of variation and selection, known to be important factors in the evolution of macroorganisms, also apply to the evolution of microorganisms, such as bacteria. McNew (10) recently has shown that organisms more virulent or less virulent on maize than the parent culture may readily be selected from pure cultures of *Phytomonas stewarti* after relatively short periods of growth. In view of the rapid rate of reproduction in a growing population of bacteria, considerable variability may be brought about in a formerly homogeneous population within relatively short periods of time, even if the rate of mutation is very low. Because of this, a bacterial population may be exceedingly plastic, capable of being molded in certain directions by the competitive selection of random mutants or variations. That certain cultures are more stable than others is shown by the failure of one of the low virulent strains to revert to high virulence after 13 passages through the resistant host (Table 4).

It has been shown that those properties enabling the organism to be more virulent on the resistant inbred line of maize OSF also enable it to be more virulent on maize inbreds, in general. But those properties enabling the organism to be more virulent on a certain grass, unrelated to maize, make it less virulent on maize. The nature of resistance of the particular grass probably was different from the nature of resistance in maize; consequently, different properties were acquired. It appears, therefore, that whether the virulence of an organism for a certain susceptible host is enhanced by successive passages through a resistant host depends on whether the nature of resistance or susceptibility is the same for both hosts.

The fact that virulence may be increased in a resistant host and decreased in a susceptible host of a particular species may have an important bearing on the rise and decline of disease epidemics in that species. At the outset of an epidemic according to Zinsser and Wilson (15) the organism is usually highly virulent. It is conceivable that, at first, a large number of susceptible host genotypes may be destroyed. But, as the organism spreads from one susceptible host to another, it may lose virulence and the epidemic may again subside. Meanwhile, the pathogen may be maintaining its virulence in some resistant host or in time may even increase in virulence in hosts of higher resistance. Given a period of time for the recurrence of susceptible host genotypes in the vicinity of resistant genotypes maintaining the highly virulent organisms, another epidemic may arise if environmental conditions become favorable for dissemination of the organism.

It is conceivable from the results obtained on the grasses that a new disease, heretofore unknown, may suddenly arise in epidemic form on a certain species of plants through evolution of the parasite in certain resistant members of that species. For example, continued passages of *Phytomonas stewarti* through Reed canary grass may eventually result in the development of a strain of bacteria that might live in equilibrium with this new host. From here it may spread to less resistant closely related varieties of this species and bring about a new epidemic.

#### SUMMARY

Effect of successive passages through resistant and susceptible host on the virulence of *Phytomonas stewarti* was studied.

Strains of *Phytomonas stewarti* were successively passed through seedlings of a highly resistant and a highly susceptible inbred line of maize. Passages also were made through seedlings of a very susceptible variety of teosinte and a series of other grasses entirely unrelated to maize.

Successive passages through the highly resistant maize host increased the virulence of the initial strains for maize. In contrast, successive passages through the susceptible maize host decreased the virulence for maize. The virulence could not be increased beyond a certain point in the resistant maize host nor decreased beyond a certain point in the susceptible host, according to the methods employed in these experiments. The parasite seemed to reach an equilibrium with its particular host environment after which further passages had no effect.

Successive passages through the susceptible variety of teosinte had an effect similar to successive passages through the susceptible maize host. Virulence was reduced for maize, as well as for teosinte.

Successive passages through highly resistant hosts unrelated to maize such as Reed canary grass, timothy, tall meadow oat grass, proso millet, and others, reduced the virulence for maize, although the organisms seemed to become more virulent for the different grasses through which they were being passed.

Cultures, highly virulent for maize, were very slimy or watery in their growth on nutrient-dextrose agar, whereas those weakly virulent for maize were of firmer growth.

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# RUST RESISTANCE IN THE GARDEN BEAN<sup>1</sup>

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## INTRODUCTION

Bean varieties were first classified as to their resistance to rust (*Uromyces appendiculatus* (Pers.) Fr.)<sup>2</sup> by Fromme and Wingard (5, 6). They found common varieties of *Phaseolus vulgaris* to be fairly constant in their disease reaction, and, in rating the varieties on a numerical scale, they used as criteria the number and size of uredia, the incubation period, necrotic reaction of the host, and relative promptness of production of telia. Harter *et al.* (12), have recorded also the relative resistance of many varieties of bean to 2 physiologic races of the fungus.

Wingard (21, 22) found that resistance to rust in certain varieties was controlled by a single gene, dominant to that controlling susceptibility. He distinguished 3 types of host reaction that were characterized respectively by no visible signs (immunity), severe flecking, and production of sori. He believed resistance was due to hypersensitiveness of the host cells to the parasite.

The investigation here reported is a study of resistance as expressed in the symptoms produced by race 1 (12) of the bean rust fungus on a number of resistant and susceptible varieties of the host. The influence of various environmental factors, especially temperature, light, and nutrition, upon the host-parasite reactions has been given considerable attention.

## MATERIALS AND METHODS

The rust culture used was secured from S. A. Wingard and was maintained on the variety Kentucky Wonder. Repeated single-sorus isolations were made on Kentucky Wonder and inoculation was frequently carried to the variety Wisconsin Refugee on which it produces only flecks. Urediniospores for inoculum were commonly stored for periods up to 2 months at 8° C. Just before inoculation they were suspended in water, filtered through cheese cloth, and sprayed on the lower side of the full-grown first leaves. Inoculated plants were kept in a moist chamber, provided with arrangements to admit diffuse daylight at 16°–20° C. for 24 hours. Readings were generally

<sup>1</sup> The writer gratefully acknowledges his indebtedness to Dr. J. C. Walker who suggested this study and under whose direction it was carried on; to Dr. E. C. Stakman for valuable suggestions and encouragement; and to Dr. A. J. Riker, Dr. W. E. Tottingham, and Dr. A. Hollaender from whom suggestions on various experimental procedures were secured. Thanks are due to Mr. B. W. Smith for his help in the preparation of the manuscript and to Mr. E. Herrling for the photographs and graphs.

<sup>2</sup> The bean rust fungus has been designated recently by Arthur as *Uromyces phaseoli typica*.



taken 10–12 days after inoculation, since, at that time, secondary sori had not developed on the most susceptible varieties, while the primary sori on the resistant varieties had matured spores. The amount of infection usually was determined by counting the number of sori (or flecks) on a square inch of leaf area. The types of infection, as described later, however, were taken as a final measure of resistance.

#### INFECTION TYPES

Since Stakman and Levine (18) proposed 6 types of infection to classify degrees of relative resistance to stem rust in wheat, the same scheme has been widely adopted in similar studies on cereal rusts. Flor (3) introduced this system, with modifications to use with dicotyledonous hosts, in his study of the physiologic specialization of *Melampsora lini* on *Linum usitatissimum*. Some peculiar divergences call for only minor modifications in adaptation of the same method to bean rust. The criteria used herein for grading the hosts as to resistance are: The success or failure of the fungus in establishing itself; the production of primary and secondary sori; and the size of sori and the time required for the maturation of spores. Early or exclusive production of teliospores is not included because, under optimum conditions, all the varieties tested produced urediospores and the production of teliospores was much modified by other factors.

The types as differentiated (Fig. 1) in the present study, are qualified, with decreasing order of resistance, as follows:

Type 0. Fungus unable to establish itself: 0o, with flecks of microscopic size; 0a, small necrotic chocolate brown blotches, with no depression at the center; 0b, larger necrotic areas up to 2 mm. in diameter, more or less circular in outline, with purplish brown margins, and depressed centers bluish at first but whitish later.

Type 1. Uredia usually small, located at the center of necrotic spots: 1a, necrotic spot of 0a type; 1b, necrotic spot of 0b type.

Type X. More than one type of infection produced by a single strain of rust on one leaf blade; spores from one type of infection capable of producing all types of infection occurring on that particular host; Xa, containing types 0a and 1a; Xb, containing 0b, 1b, and 2 or 3.

Type 2. Uredia small, 150–300  $\mu$  in diameter; often not extending through the leaf; with or without merely perceptible chlorotic ring; secondary sori formed very slowly.

Type 3. Uredia mid-size, 250–500  $\mu$  in diameter; often associated with a chlorotic halo, especially on young vigorous leaves; secondary sori produced 8 to 10 days after the appearance of the primary sori.

Type 4. Uredia large, secondary sori produced 6 to 8 days after the appearance of the primary sori.

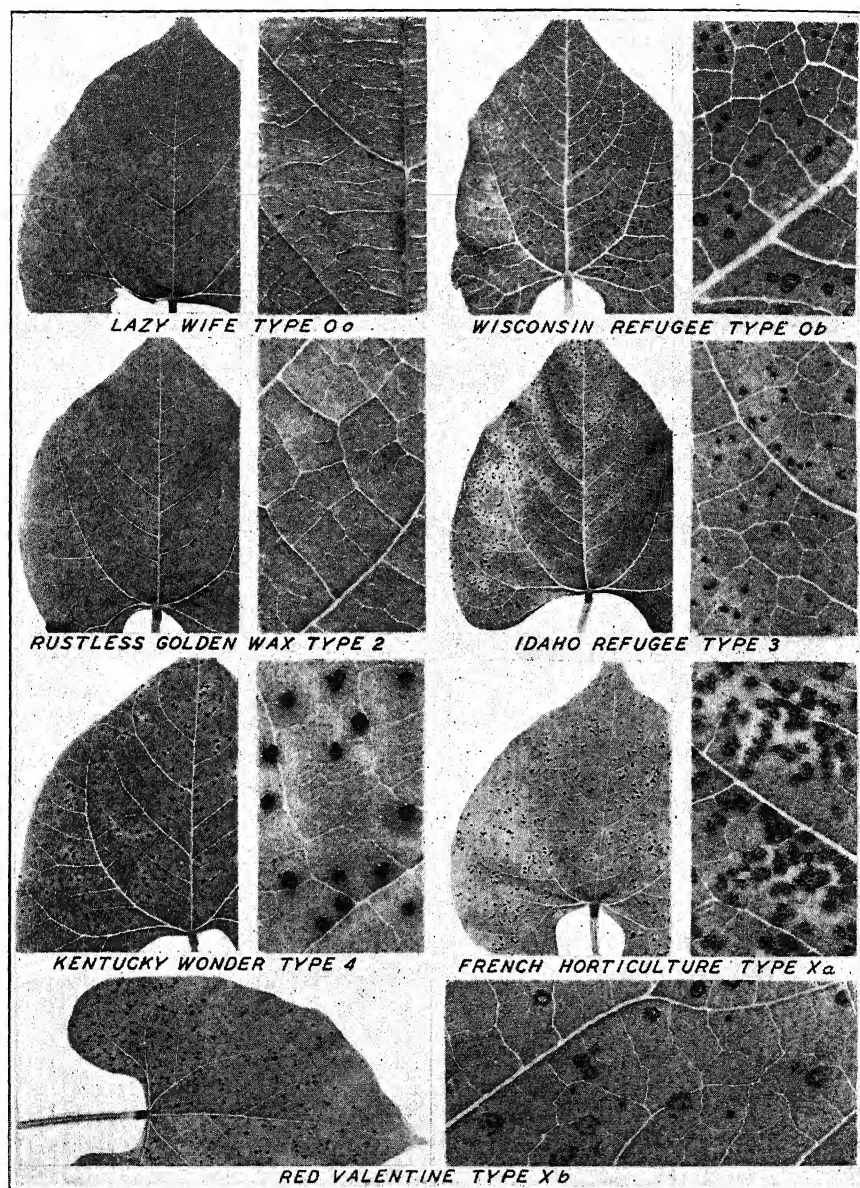


FIG. 1. Various types of reaction by different varieties of bean (*Phaseolus vulgaris*) to physiologic race 1 of the bean rust fungus. Varieties and type classes are indicated. (See further explanation in the text.)

The varieties of bean tested are classified as follows:

Type 0o. Lazy Wife.

Type 0a. This type of infection has been observed only in Xa type of reaction.

Type 0b. Early Rogers Refugee, Early Stringless Refugee, Hodson Wax, Idaho Refugee (in part), Stringless Refugee Wax, Wisconsin Refugee, Scarlet Runner (*Phaseolus multiflorus*).

Type 1a and 1b. These types of infection have been observed only on Xa and Xb hosts.

Type Xa. Erntebringer, French Horticulture, Golden Wax, Kentucky Wonder Wax, Mulstopper, New Stringless Green Pod, Phenomenon (in part), U. S. No. 3, U. S. No. 4, and Zeppelin (in part).

Type Xb. Asgrow Stringless, Asgrow Stringless Valentine, Brittle Wax, Curries Rust Proof Black Wax (in part), Improved Golden Wax, Improved Kidney Wax, White Seeded Kentucky Wonder, Pencil Pod Black Wax, Red Valentine, Sure Crop Black Wax, Wardwell's Kidney Wax, Webber Wax, and White Seeded Refugee Wax.

Type 2. Landreth Stringless, Rogers Stringless Black Valentine, and Rustless Golden Wax.

Type 3. Black Valentine, Bountiful, Curries Rust Proof Black Wax (in part), Davis White Wax, Full Measure, Giant Stringless Green Pod, Idaho Refugee (in part), Longfellow, Red Kidney, Refugee Wax, Ruby Dwarf Horticulture, Unrivalled Wax, U. S. No. 1; Fordhook Bush Lima (*Phaseolus lunatus*).

The above classification is based on 4 inoculation experiments at 20°-24° C. It is to be noted that several varieties were included in each of two groups. This is due to the fact that such varieties are not pure lines for a given type or contain two distinct lines. It is believed, however, that the classification of commercial varieties on the basis of type of infection is much more logical and useful than rating on the basis of amount of infection.

#### EFFECT OF TEMPERATURE ON TYPE OF INFECTION

The relation of temperature to the development of several cereal rusts has been reviewed by Johnson (13), Gordon (8), and Melander (14). In general the influence is greatest on the intermediate or mesothetic varieties of the host. In the present study the test of the effect of temperature on host reaction was conducted in the greenhouse at Madison, Wisconsin, between late September and February, when the low, outdoor temperature enabled satisfactory control of temperatures in the greenhouse. Plants were grown, prior to inoculation, around 20° C. and inoculated with spore-suspensions when they reached a desirable stage of development. After 24 hours in the moist chamber, at about 18° C., they were grouped and distributed into 4

compartments with temperatures maintained at 16°, 20°, 24° and 28° C., respectively. The varieties used were Lazy Wife (type 0o), Wisconsin Refugee (type 0b), French Horticulture (type Xa), Red Valentine (type Xb), Rustless Golden Wax (type 2), Idaho Refugee (type 3), and Kentucky Wonder (type 4).

The length of the incubation period varied in all cases inversely with the rise in temperature. The type of infection did not change with temperature in Kentucky Wonder, Idaho Refugee, French Horticulture, Wisconsin Refugee, nor Lazy Wife. On Rustless Golden Wax (type 2) it was normal at 16–20°, but was changed to type X, at 24° to 28°. The greatest fluctuation of infection type occurred in the case of Red Valentine, a type Xb variety in which 0b, 1b, and 3 types occur commonly on the same leaf blade. The relative percentage of infections in each type at each temperature was determined by counting and classifying the sori or flecks on a square inch of leaf surface from each temperature. This involved counts of 600 or more sori or flecks in each case. The results of 3 experiments are given in table 1.

TABLE 1.—*Relation of temperature to infection types on Red Valentine variety*

Temperature °C.	Experiment No.	Infection type		
		0b	1b	3
		<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
16	1	0.2	66.3	33.5
	2	16.6	62.5	20.9
	3	0.2	73.4	26.4
20	1	7.1	81.1	11.8
	2	37.5	61.5	1.0
	3	12.7	80.9	6.4
24	1	23.3	69.0	7.7
	2	31.3	67.4	1.3
	3	8.0	81.6	10.4
28	1	6.5	57.2	36.3
	2	17.0	62.9	20.1
	3	2.3	47.0	50.7

A majority of the infections, with one exception, were of 1b type. The largest number of 0b infections resulted at 20° and 24°, while the largest number of type 3 infections occurred at 16° and 28°.

The results indicate clearly that in varieties that are either highly resistant or highly susceptible to rust the type of reaction of the host to infection is influenced very little by variation in temperature. However, in a mesothetic host such as Red Valentine, in which a range of infection types occurs, the relative proportion of each type of reaction is modified. It appears that the typical X reaction occurs around 20° C., which is near the optimum for

the host. At lower temperatures the reaction between host and fungus is far less violent, as shown by a very narrow necrotic zone in the 1b sori, whereas, at higher temperatures, this zone occupies most of the spot. The green center delimited by this band is much larger at 16° C. and the sori contain many more spores than at higher temperatures, although they are mostly teliospores. This, together with the fact that the relative number of type-3 sori is larger at 16°, indicates that the X host is relatively more susceptible at that temperature. Similarly, at 28° the increase in type-3 sori indicates increase in susceptibility, although the appearance of type-1b sori is not changed from that at 20° and 24°. This may be due to the fact that the leaves age faster at 28° and the reactivity of the host cells is impaired by the early onset of senescence.

#### EFFECT OF LIGHT UPON INFECTION

The influence of light on rust development has received much attention and results often have appeared to conflict. Comprehensive discussions can be found in papers by Forward (4) and Melander (14). Most workers agree that the absence of light or reduction of light intensity causes a prolongation of the incubation period. Hart and Forbes (11) found that the effect on the initiation of infection varies with the rust in question.

Waters (20) subjected corn plants inoculated with rust to several days of darkness and found that necrosis was produced around the sorus on the susceptible host. Johnson (13) diagrammed the shifting of X-type infection to type 4 in stronger light and to type 0 by weak illumination. Forward (4) induced "hypersensitiveness" on the susceptible host by subjecting wheat plants infected by *Puccinia graminis tritici* to continuous darkness. This effect was absent if the plants were exposed to light for a short period every day or if the leaves were detached before subjecting them to darkness. When the leaves showing abnormal reactions were returned to light early enough, secondary sori developed with normal size. Bever (2) changed type-4 infection of stripe rust (*Puccinia glumarum tritici*) to 0 type by exposing the plants to a light period longer than 12 hours a day. Shorter-day periods did not, however, shift the reaction in the other direction. Melander (14), working on *P. graminis tritici*, did not modify the infection types by variation in light treatment.

In order to study the effect of light on bean-rust infection, an inoculation chamber was divided into a light and a dark compartment. The light compartment was exposed to the normal greenhouse daylight and was illuminated at night by a 200-watt Mazda lamp. The dark chamber was supplied with a small fan (about 150 r.p.m.) to minimize other variations. It enabled air circulation to equalize the temperature and to prevent the accumulation of CO<sub>2</sub> which, as pointed out by Scarth (16), might cause the stomata to

open. All 4 groups of plants were kept in either of these 2 chambers in order to maintain similar conditions during the 6-day period.

Plants of Kentucky Wonder were grown in 4-inch pots with 3 plants in each. They were divided into 4 groups, consisting of 6 pots in each group. The first group was subjected to darkness for 48 hours before inoculation. The second lot was darkened for 48 hours after inoculation. The third group was kept dark during the 48-hour inoculation period. The fourth group was kept in the light chamber throughout. During that period water was always amply supplied to the soil. A parallel experiment was run without operation of the ventilating fan in the chamber. Results are summarized in table 2. It is evident that light is essential for bean-rust infection, and that lack of ventilation modified the effect considerably.

TABLE 2.—*Effect of light upon the amount of bean-rust infection*

Period of darkness	Number of sori per square inch			
	In ventilated chamber			In nonventilated chamber
	Exp. 1	Exp. 2	Exp. 3	Exp. 1
48 hrs. before inoculation .....	114.8	55.8	169.4	58.1
48 hrs. during inoculation .....	0.5	1.6	37.9	36.0
48 hrs. after inoculation .....	105.7	.....	.....	.....
None .....	63.5	63.0	210.1	55.2

The influence of light intensity on infection was studied by the use of chambers to which light gained access only through the tops. The intensity of light in the chamber was varied by providing different numbers of layers of 16-mesh wire screen over the top. The intensity of light in each chamber was measured by a photometer and the relative proportions of light in each was thus computed. In all varieties tested the length of the incubation period varied inversely with light intensity.

Insofar as the type of infection was concerned, the highly susceptible and the highly resistant varieties were least affected. On Wisconsin Refugee (type 0b) typical flecks were found until the light became so weak that the leaf turned yellow before any typical symptoms appeared; then the infections were manifest as green specks. The rust developed very slowly on Rustless Golden Wax (type 2) when the light intensity was less than one half that of the open chamber, and the spores produced were found to be predominantly teleutospores, although no change of type was definitely shown. The pustules on Idaho Refugee (type 3) were gradually reduced with the reduction of light and the type of infection fell to type 2 when the light was

reduced to less than one third of the open chamber. On the Kentucky Wonder (type 4) a certain amount of type 1b infections were observed. The necrotic zones around the sori were very narrow and the pustules were of considerable size. Superficially, they resembled the type 1b infection on Red Valentine plants kept at 16° C. The proportion of such infections varied inversely with the light intensity. In one of the experiments the percentages of such type of infection were 0.00 in the open chamber, 2.07, 9.81, and 14.27 when the light was reduced to 1/1.5, 1/2, and 1/3, respectively.

The most interesting change took place in the variety Red Valentine in which 3 types of infection, type 0b, 1b and 3, normally occur. The proportion of type-3 infections was found to increase with the reduction of light intensity, within the limit where leaves survived long enough to give distinct symptoms. The types 0b and 1b were proportionally reduced. This general tendency was shown by all the 4 experiments performed.

The increase in proportion of the type-3 infection, as an indication of more congenial relationship between the host and the parasite, with the reduction of light intensity had been well duplicated in similar experiments. The occurrence of such a phenomenon can reasonably be interpreted if the necrosis be considered as the result of antagonism between the host and fungus. Under weak light condition the host is weak as evidenced by the pale green coloration of the leaf; and the fungus develops slowly, as indicated by the long incubation period, so that the interaction between these 2 organisms is likely to be slow and mild. As the result of this, necrosis will occur to a less extent and an increased development of the fungus will be permitted.

The occurrence of necrosis on the type 4 host (Kentucky Wonder) under weak light condition seems to be contradictory to the effect of the same treatment on the X-type host discussed before. However, this can be interpreted by assuming that this type of host, under normal conditions, can tolerate the toxin produced by the fungus to a much greater extent than other hosts; but when the host tissue is so weakened by adverse environment, it is no longer able to stand the toxin, and death is the result. This supposition seems to have some support since this variety is more sensitive to reduced light than some other varieties tested, and patches of necrosis often appear as a result of this treatment even without rust infection.

#### EFFECT OF MINERAL NUTRIENTS OF THE HOST ON RUST INFECTION

A considerable amount of work has been done on the effect of nutritive elements on disease resistance. The more important literature concerning the effect of fertilizer on rust development had been reviewed and discussed by Hart (10) and Gassner and Hassebrauk (7). Nitrogen has been found to favor disease development, while potassium has the opposite effect. Host

varieties that have a mesothetic reaction respond much more readily than the extremely susceptible or resistant ones. The balance of different elements also has been considered to be of greater importance in influencing disease incidence than the kind and amount of element.

The experimental work on the effect of salt nutrition on rust reaction of the bean varieties was done with sand and water culture. After a comparative study of the growth of bean plants in solutions prepared according to several formulae, the following modification of Shive's solution was adopted:

$\text{Ca}(\text{NO}_3)_2$  and  $\text{KH}_2\text{PO}_4$ , 9 millimols each;  $\text{MgSO}_4$ , 6 millimols; Iron ammonium citrate, 0.001%; boric acid, 0.8 ppm.

In case of sand cultures, the addition of boric acid was unnecessary because the amount of boron in the sand, as an impurity, was large enough to maintain normal growth. Manganese was not added, except as an impurity in the chemicals or in the sand.

In cases where an excess of nitrogen was desired 18 millimols of  $\text{NH}_4\text{NO}_3$  were added to the full solution to make the nitrogen content 3 times as great; and in the nitrogen-deficient series, 8 millimols of  $\text{Ca}(\text{NO}_3)_2$  were replaced by 4 millimols of  $\text{CaCl}_2$  and 4 millimols of  $\text{CaSO}_4$ . The entire replacement by  $\text{CaCl}_2$  or  $\text{CaSO}_4$  was purposely avoided to prevent the possible toxic action of  $\text{CaCl}_2$  in high concentration on the one hand and an oversupply of sulphur on the other. In the case of excess phosphorus 18 millimols of  $\text{NaH}_2\text{PO}_4$  were added to the full solution to triple the phosphorus content, and in phosphorus-deficient series 8 millimols of  $\text{KH}_2\text{PO}_4$  were replaced by KCl. Excess of potassium was obtained by adding 18 millimols of KCl and deficiency of potassium was secured by replacing 8 millimols of  $\text{KH}_2\text{PO}_4$  by  $\text{NaH}_2\text{PO}_4$ . The pH of the resultant solutions varied only between 4.8-5.0, which is not large enough to interfere with the plant growth.

The varieties used were Wisconsin Refugee (type 0b), Red Valentine (type Xb), Rustless Golden Wax (type 2), Idaho Refugee (type 3), and Kentucky Wonder (type 4). The plants were inoculated when the first leaves had almost fully expanded. In general, excess nitrogen favored disease development, while excess potassium augmented resistance, both in water and in sand cultures. Low potassium usually resulted in a larger number of pustules. Neither high nor low phosphorus had any consistent effect.

No perceptible effect upon the type of infection was noted in highly resistant nor highly susceptible varieties. Red Valentine, the mesothetic variety that was decidedly influenced by variation in temperature and light, was influenced also in its reaction to the parasite when the nutritive elements were varied. The relative amount of type 3 infections invariably increased with decrease in vigor of the host, namely in the nitrogen-deficient and



potassium-excess series. In nitrogen-high and potassium-low plants, where the leaves were greener and remained longer on the plants, the percentage of type-3 sori was consistently lower and the type-1b infections consistently higher than in the controls. It would appear, therefore, that there is a definite correlation between the vigor level at the time of inoculation and the amount of infection. In Red Valentine the type of infection also seems to correlate with vigor. When senescence is delayed, the proportion of flecks (type 1b) is increased, while, when senescence is hastened, the proportion of pustules (type 3) increases.

#### THE EFFECT OF SOME MINOR ELEMENTS ON BEAN RUST

The possible influence of minor elements on the amount and type of infection was investigated by their addition to the standard nutrient solution in definite quantities. The amount of infection was not influenced by variation in the amount of either boron or lithium added; nor was the type of infection changed, except that, in Red Valentine, the proportion of type 3 infections was increased when 0.16 millimols of boron were added. The addition of small amounts of zinc (2 millimols or higher) was distinctly detrimental to the host and the number of infections per unit area was correspondingly reduced. No definite effects were secured with addition of small amounts of germanium. In this series the type of infection did not appear to be affected.

#### RELATION OF AGE OF HOST TISSUE TO INFECTION

Increase in resistance to rusts with increase in age of the host tissue has been observed commonly. This was not the case in bean rust. The effect on length of incubation was studied with Kentucky Wonder (type 4) and with Wisconsin Refugee (type 0b). These were sown at 4-day intervals. When the first leaves of plants in the fifth seeding had completely unfolded, the color of those on plants of the first seeding had begun to fade. All were inoculated at this time. The symptoms appeared on the youngest leaves about 1 day earlier than those on the oldest ones and there was some but small decrease in the amount of infection noted with advance in age of the tissue in both varieties.

The effect of age of tissue on the type of reaction was studied on Kentucky Wonder (type 4), Idaho Refugee (type 3), Rustless Golden Wax (type 2), Red Valentine (type Xb), French Horticulture (type Xa), Wisconsin Refugee (type 0b), and Lazy Wife (type 0o). The effect upon each was, briefly, as follows:

Kentucky Wonder.—The lower leaves were pale green and the sori were smaller. The young leaves bore normal sori. Both, however, were type 4.

Idaho Refugee.—Lower leaves pale green, with many smaller sori; upper leaves vigorous, with sori surrounded by prominent chlorotic halos. The classification of reaction remained unchanged.

Rustless Golden Wax.—The reaction on the older leaves was typically 2, while that on the younger leaves was Xb, although the number of type-0b and 1b infections was comparatively small.

Red Valentine.—Lower leaves pale green, showed only type-3 infections. On the full-grown leaves, the reaction was typically Xb, while the reaction on the third leaves was largely type 0b.

French Horticulture.—Older leaves, paler in color. Type 1a infections were more abundant on the older leaves, while type 0a infections were predominant on the young ones.

Wisconsin Refugee.—Normal reaction took place, although the size of flecks was larger on the first leaves.

Lazy Wife.—Microscopic flecks of similar size occurred on both the old and young leaves.

The results of a second experiment with Rustless Golden Wax are given in table 3.

TABLE 3.—*The effect of age of the host tissue on the type of disease reaction in Rustless Golden Wax*

Age of tissue (days after unfolding)	Number of leaves observed	Percentage of leaves showing different disease reactions		
		Type 0b	Type Xb	Type 2
1 .....	27	18.5	51.9	29.6
5 .....	29	0.00	31.3	68.7
9 .....	30	0.00	0.00	100.00
13 .....	20	0.00	0.00	100.00

A more detailed analysis was made of Red Valentine, in which the number of spots of each type was counted on the first leaves of different ages and the proportion of them expressed in percentages (Table 4).

TABLE 4.—*The effect of age of host tissue on the type of infection in Red Valentine*

Age of leaf (days after unfolding)	Percentage of type of infection		
	Type 0b	Type 1b	Type 3
0 .....	54.6	44.7	0.7
3 .....	56.5	43.0	0.5
6 .....	23.8	36.6	39.6
9 .....	21.3	40.6	38.1
12 .....	3.0	13.5	83.5
15 .....	0.6	0.5	98.9
18 .....	0.2	0.2	99.6
21 .....	0.4	0.5	99.1

This tendency of shifting of the proportion of types of infection was repeatedly shown in other experiments.

The decrease of the amount of infection with the age of the tissue seems to indicate that some mechanism had developed either to exclude certain infectious hyphae or to prevent development of some fungi that had entered. On the other hand, the violence in host-parasite interaction, which determined the establishment of the fungus and the eventual production of reproductive bodies, probably is governed by a quite different mechanism. This point is brought out by the increase of the proportion of fertile sori with the age of host tissue, while the same host condition reduced the amount of infection. The aging of the host tissue seems to alter its cellular mechanism, so that a congenial relationship between the host and the fungus is made possible.

The mechanism behind this change is obscure. The best-known change in aging of tissues is dehydration, that is, the condensation of organic compounds into less hydrated forms, as sugars into starch, amino acids into proteins, etc. This, however, can hardly explain the situation. Works on other diseases indicate that more hydrated forms of organic compounds are better food material for the fungus and the tissues containing them are more susceptible. Moreover, the same parasite develops much better on young tissues on the susceptible hosts of the same species. One suggestion is that the host cells on aging gradually lose either the ability to respond as violently to the stimulus of the intruder or the ability to produce substances that are toxic to the fungus.

#### DISCUSSION

The results presented seem to justify the general conclusion that the part played by the host in determining the type of infection is not less than the part of the parasite or *vice versa*. In the first place, the different types of infection are a result of varietal differences in host as well as the differences in the strains of parasite. When the interaction of host and parasite is such that the "toxin" and "antibody" are produced in large quantities, the death of both tissues is immediate and results in flecking. In case one or both of these substances are produced in small amount or the tolerance of either the host or the parasite or both is high, the host-parasite relationship is more congenial. The situation in the intermediate types of infection probably represents gradations between these extremes.

Secondly, the host-parasite balance varies in stability. In the most resistant and in the most susceptible reactions, the mechanism is so well fixed that the disease expression is not readily affected by varying the environment, while it exists in a more readily modified stage in the intermediate (X) type. The change of type of infection on the X-type host by different treat-

ments probably is the result of interference, which shifts the host-parasite balance in one direction or another. When the host is weak the fungus is not much antagonized, so that the latter will be able to develop better, while the fungus may produce less "toxin" under such conditions, so that only a small portion of infected host tissue will be killed. The reverse may be true when the host tissue is vigorous.

Another point suggested by the results presented is that the amount of infection and the development of the fungus are controlled by independent mechanisms. A larger amount of infection does not correlate positively with the more congenial type of infection, and, in fact, their relationship is just opposite to this.

The resistance to rust found in beans has been claimed by Wingard (22) as due to hypersensitiveness. This type of resistance was first intensively investigated by Ward (19) in brome rust caused by *Puccinia dispersa*. By cytological methods he studied the host-parasite relationship in both susceptible and resistant hosts. In 1902 (19) he published 4 types of disease reactions, in 1 of which he thought the destructive action of the infecting tubes had killed the cell too rapidly. "The affected patch thus appears corroded, and since the dead cells are unsuitable as a medium for further growth of the mycelium the parasite dies." This idea was favored by Stakman (17) in interpreting his cytological results in the studies on stem-rust resistance in wheat, and he introduced the term "hypersensitiveness" to denote such a phenomenon, although "without any implication as to the exact physiological nature of the phenomenon."

Allen (1), in discussing her cytological results in the study of the infection of susceptible and resistant varieties of wheat by *Puccinia graminis tritici*, refuted the starvation theory and wrote: ". . . there seems little room in this particular case for the starvation theory of immunity tentatively discussed by Ward, Marryat, Gibson, Spinks, and others. At every point of entry into a host cell the fungus is either killed back or driven back for a short distance. When the reaction of the host is somewhat deferred, the fungus makes a haustorium, and it evidently extracts food from the host—enough at least to let it grow on to new cells—and there is no evidence that this food is of an unsuitable nature. The failure of the fungus is due not so much to lack of proper food in the host as to a specific reaction set up there which destroys the fungus." She pointed out that the development of the fungus, in the resistant host, ". . . is similar until a small haustorium is formed, which, either by its presence, or, as is more likely, by secreting some substance in the host cell, set up chemical reactions within that cell, causing its collapse and death. . . . One or more of the substances formed in the host cell diffuse into the haustorium, killing it and causing collapse of the mother cell and the death and plasmolysis of the hyphae back of it for some

distance. . . . Immunity is due to definite antagonistic chemical interactions between host and parasite."

Nusbaum (15) considered that since "the antagonism against the apple rust fungus is already present in the make-up of the host-cell protoplasts or is induced by the parasite and presents a counterreaction against the intruder is open to question. Cytological evidence cannot settle this point." He, however, thought that "the failure of the fungus to establish itself could not be attributed to hypersensitiveness of the host cells, resulting in starvation of the obligate parasite. Death and collapse of the fungus preceded injury to the host cells."

The present study, although insufficient to warrant any definite explanation of the mechanism of this type of resistance, revealed the inadequacy of attributing the mechanism entirely to either the host or the parasite. It is not only a host action, either the passive starvation or the active killing of the fungus, because the fungus often develops to some extent, even in the type-0 infection; and in type-1 infection the necrosis, in most cases, appears after a considerable amount of mycelium has developed. The latter type can hardly be considered attributable to a different mechanism from that determining the former one, because all gradations between these 2 types are readily observable. Also, the fungus can hardly be solely responsible, for, on the tissue of different ages in the X-type host or on plants supplied with different ratios of nitrogen to potassium, the development of the fungus is apparently of the same rate and amount; yet, the proportion of the types of infection varies considerably, and the shifting corresponds closely to the degree of change in the host condition. It seems to be most reasonable to assume that both host and parasite play an active part in the antagonism and the priority of the death of the parasite, or that the host varies from case to case, depending upon the relative production of "toxin" and "antibody" and relative resistance of both the host and parasite tissues.

#### SUMMARY

Types of infection in bean rust and the factors affecting their development have been studied.

From 50 bean varieties, 5 major types of infections are distinguished, based on the extent of necrosis on the one hand and the amount of sporulation on the other. These types are, with descending order of resistance, 0, 1, 2, 3, and 4. Type 0 is subdivided into 0o, 0a, and 0b, and 1 into 1a and 1b, according to the extent and type of necrosis. 0, 1, and 2 are considered as resistant types, 3 and 4 susceptible ones, and the plants that show both susceptible and resistant types of infection are mesothetic and designated as the X-type, which is also divided into Xa and Xb types.

Temperature ranging from 16° to 28° C., does not change the type of reaction, except that the type-3 infection on the X-type host is increased, in proportion, by both high and low temperatures. Low temperature prolongs the incubation period at the same time.

Light is essential during the infection period for successful entrance of the fungus. Reduction in light intensity prolongs the incubation period. Beyond a certain extent it induces necrosis in type-4 infection, and increases the proportion of type-3 infection on X-type hosts. It has no effect on the type of infection otherwise.

Excess of nitrogen increases the amount of infection per unit area of the leaf and *vice versa*. Potassium has an entirely opposite effect. The effect of phosphorus is not definite. The variation in nutrient supply tested does not change the type of infection, except that low N/K increases type-3 infection on the mesothetic host.

Lithium, boron, zinc, and germanium affect neither the amount nor the type of infection until their concentration is high enough to cause abnormal growth of the host. On the X-type host any condition that retards maturation is accompanied by the increase of type-0 infection, while that that hastens senescence encourages the development of the fungus. The amount of infection also is generally decreased by the latter.

Aging of the host tissue, beyond a certain limit, reduces the amount of infection. It does not affect the type of infection materially on most varieties, but the proportion of type 3 on the mesothetic host is increased with age until it entirely replaces the other 2 types of infection.

It is suggested that the nature of this protoplasmic resistance is the result of a combined action in which both the host and the parasite take part. The death of the host cell and the parasite is probably due to the action of "toxins" and "antibodies" produced by the respective organisms.

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# INOCULATION OF SOME ECONOMIC PLANTS WITH PHYTOPHTHORA CACTORUM AND P. CITROPHTHORA<sup>1</sup>

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*Phytophthora citrophthora* (Sm. and Sm.) Leonian has a limited host range and probably is found in nature only on *Citrus* spp. *Phytophthora cactorum* (L. and C.) Schröt., on the other hand, is omnivorous and has been reported on numerous hosts. This paper describes some artificial inoculations (Table 1) with the two organisms on a number of different trees and shrubs.

Fawcett (2) has described successful inoculations on *Persea americana* (avocado) with cultures of *Phytophthora citrophthora* from lemon and with an isolate from avocado, described by Barrett (1) as *Phytophthora cactorum*. Inoculation with the avocado strain of *P. cactorum* on citrus stems was negative, while that with *P. citrophthora* was positive. Smith and Smith (6) studied a disease of deciduous nursery stock and fruit trees (peach, plum, apricot, and cherry). They made isolations of different strains of *Phytophthora* and reported having some strains of *P. cactorum* and others that resembled the *P. citrophthora* type. Gravatt (3, p. 333) found that seedlings of *Pinus* were extremely susceptible when inoculated with *Phytophthora citrophthora*, but this organism never had been found occurring naturally in coniferous seedbeds. Klotz and Fawcett (4) investigated the relative resistance of varieties and species of citrus to *P. citrophthora*. Tucker (7) made successful inoculations with *P. citrophthora* on fruits of eggplant, tomato, and apple, but those on the stems of various plants gave only negative results. Smith and Barrett (5) produced lesions on the trunk of *Juglans californica* with *P. citrophthora*.

*Phytophthora cactorum* can attack a number of plant species, some of which are listed by Tucker (8), although he was unable to infect *Citrus* spp. with any of the various strains of *P. cactorum* that he used (7).

The method of inoculation consisted in placing a pure growth of the mycelium from glucose-potato agar into cylindrical wounds in the bark of plants growing in the open. The culture of *Phytophthora cactorum* used in the inoculation was isolated from *Juglans regia*; that of *P. citrophthora* was isolated from lemon.

The wounds were made with a 7-mm. cork borer. After the insertion of the fungus, the excised piece of bark was replaced and the wound was

<sup>1</sup> Paper No. 382, University of California Citrus Experiment Station and Graduate School of Tropical Agriculture, Riverside, California.



TABLE 1.—Artificial inoculations in woody stems with *Phytophthora* spp. in series of fives and one control

Species inoculated	<i>P. cactorum</i>		<i>P. citrophthora</i>	
	Number positive	Maximum radius of affected area, in mm.	Number positive	Maximum radius of affected area, in mm.
<i>Annona cherimola</i> .....	5	10	1	10
<i>Carya pecan</i> .....	5	10	4	10
<i>Castanea</i> sp. ....	4	25	5	45
<i>Ceratonia siliqua</i> (carob) .....	5	10	5	20
<i>Cedrus deodara</i> .....	4	20	4	25
<i>Cupressus macrocarpa</i> .....	0	0	0	0
<i>Cupressus lusitanica</i> .....	0	0	0	0
<i>Chamaecyparis lawsoniana</i> .....	0	0	0	0
<i>Citrus</i> (lemon fruits) .....	Positive rots		Typical rots	
<i>Citrus limonia</i> .....	2	4	2	120
<i>Citrus paradisi</i> .....	3	3	4	39
<i>Citrus sinensis</i> .....	3	5	3	48
<i>Cydonia oblonga</i> .....	5	20	4	20
<i>Diospyrus kaki</i> .....	1	5	3	10
<i>Eucalyptus</i> sp. ....	3	10	5	15
<i>Eriobotrya japonica</i> .....	5	25	5	30
<i>Feijoa sellowiana</i> .....	0	0	0	0
<i>Ficus</i> (Mission fig) .....	5	25	5	35
<i>Frazinus</i> sp. ....	4	6	4	10
<i>Hakea</i> sp. ....	4	25	5	20
<i>Juglans californica</i> .....	5	150	5	130
<i>Juglans formosa</i> .....	2	15	...	...
<i>Juglans hindsii</i> .....	4	100	1	25
<i>Juglans insularis</i> .....	2	10	...	...
<i>Juglans regia</i> .....	5	65	2	80
<i>Juglans nigra</i> .....	5	100	4	80
<i>Juglans pyriformis</i> .....	5	45	3	10
<i>Juglans sieboldiana</i> .....	3	30	2	20
<i>Juniperus procera</i> .....	3	8	3	10
<i>Olea europea</i> .....	3	7	2	10
<i>Persea americana</i> .....	5	5	5	20
<i>Psidium guajava</i> .....	4	20	0	0
<i>Photinia arbutifolia</i> .....	5	30	5	25
<i>Pinus muricata</i> .....	0	0	5	25
<i>Pinus coulteri</i> .....	1	5	5	10
<i>Populus fremonti</i> .....	5	45	5	60
<i>Prunus avium</i> .....	5	120	5	150
<i>Prunus armeniaca</i> .....	5	100	5	40
<i>Prunus communis</i> .....	4	100	5	60
<i>Prunus ilicifolia</i> .....	4	10	4	25
<i>Prunus</i> (Methley plum) .....	4	40	5	40
<i>Prunus nune</i> .....	5	65	5	35
<i>Prunus persica</i> .....	5	50	3	35
<i>Pyrus communis</i> .....	5	20	5	10
<i>Pyrus malus</i> .....	3	5	3	10
<i>Quercus lobata</i> .....	5	20	5	30
<i>Rosa laevigata</i> .....	1	5	1	5
<i>Thuja orientalis</i> .....	0	0	0	0
<i>Ulmus</i> sp. ....	4	8	5	10

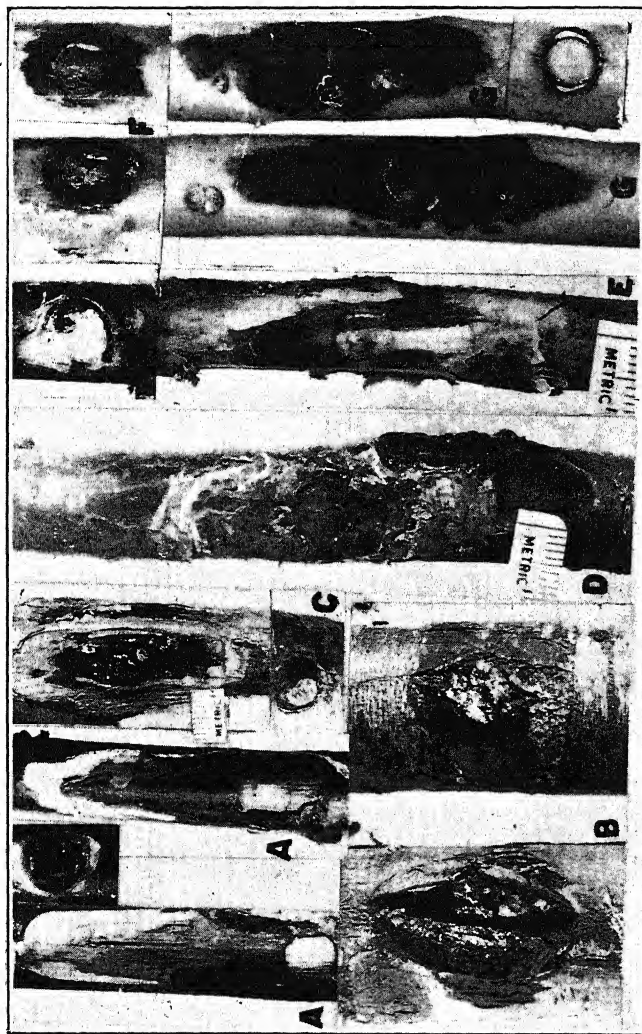


FIG. 1. Lesions produced by artificial inoculation with species of *Phytophthora*. A. Lesions produced on *Castanea* sp. by *P. citrophthora*; control in center. B. and C. Lesions on *Eucalyptus* sp.: B produced by *P. citrophthora*; control at right; C by *P. cactorum*; control below. D and E. Lesions on *Cedrus deodara*; D produced by *P. cactorum*; E, by *P. citrophthora*; control above. F and G. Lesions on *Persea americana*; F produced by *Phytophthora cactorum*; G by *P. citrophthora*; control at right.

wrapped with nurseryman's tape. One control was made for each series of inoculations and was situated on the same stem several inches below.

The aerial lesions were measured 90 days after inoculation and showed that each of the organisms was capable of infecting species of plants widely separated botanically (Table 1). Among the more susceptible plants were 4 species of *Juglans*, 12 species of the Rosaceae, including 7 species of *Prunus*. Some of the other plants infected (Fig. 1) were: *Cedrus deodara*, *Castanea* sp., *Ceratonia siliqua* (carob), *Fraxinus* sp., *Persea americana*, *Eucalyptus* sp., and an evergreen species of *Ulmus*. In some of the inoculations listed in table 1 the lesions are small; and, under natural conditions, it is probable that these slightly infected hosts would rarely, if ever, become diseased.

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## INDOLEACETIC ACID GALLS OF A SECONDARY TYPE

NELLIE A. BROWN AND F. E. GARDNER

(Accepted for publication Aug. 24, 1937)

In a recent article in this JOURNAL<sup>1</sup> the production of galls on Kidney beans by means of indoleacetic acid was described and illustrated. Subsequent papers<sup>2,3</sup> describe the anatomical development of these overgrowths.

A type of response noted at the time of the original paper, photographed, and held awaiting further evidence of similar growths in subsequent experiments, was that of secondary galls.

Crown galls formed at the inoculation point where *Bacterium tumefaciens* is inserted are generally called primary galls. The galls on other parts of the stem some distance from the original point of inoculation which develop later and where no organism has been inserted are designated as secondary galls.

An analogous situation frequently occurred with the overgrowths produced by indoleacetic acid. In addition to the primary galls induced by indoleacetic acid-lanolin mixture, secondary galls also appeared at some distance from the point of application.

The number of secondary galls on a given bean plant may vary from 1 to 15. Figure 1 shows a plant that bore 15 secondary galls, some of which were cut from the photograph in trimming it for illustration. Figure 2 shows 3 other bean plants, in the same experiment, that likewise produced secondary galls.

These secondary galls are not produced through any inadvertent carrying of the indoleacetic acid-lanolin mixture to other parts of the plants by insects or watering. They arise, as their deep-seated initial development shows, through some internal condition. It is possible that there is an overabundance of growth substance brought about in some bean plants when the indoleacetic acid-lanolin mixture is added to plants already well-supplied with their own growth substance. This overbalance the plant is not able to adjust and the number of secondary galls appearing may represent the extent to which the maladjustment of the plant is subjected. It is possible also that an over supply of growth substance may occur in a plant, with accompanying gall formation, where no indoleacetic acid or the like has been added.

<sup>1</sup> Brown, Nellie A., and F. E. Gardner. Galls produced by plant hormones, including a hormone extracted from *Bacterium tumefaciens*. Phytopath. 26: 708-713. 1936.

<sup>2</sup> Kraus, E. J., Nellie A. Brown, and K. C. Hamner. Histological reactions of bean plants to indoleacetic acid. Bot. Gaz. 98: 370-420. 1936.

<sup>3</sup> Hamner, K. C., and E. J. Kraus. Histological reactions of bean plants to growth promoting substances. Bot. Gaz. 98: 735-807. 1937.

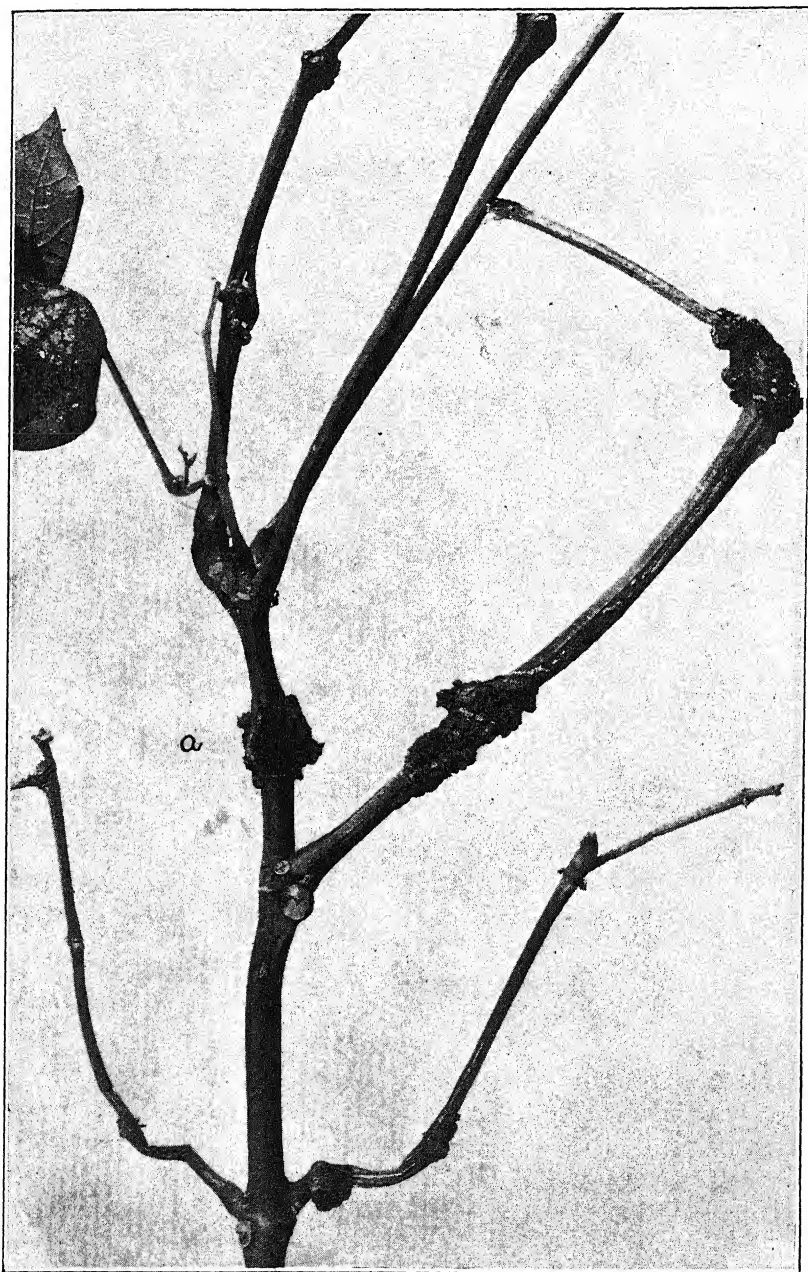


FIG. 1. Primary and secondary hormone galls on Kidney bean stems. Stem smeared with indoleacetic acid-lanolin mixture in a ring at *a*, 11-9-36. The other galls on the stem are secondary, the stem at those places received none of the indoleacetic acid-lanolin mixture. Photo. 1-6-37. Natural size.

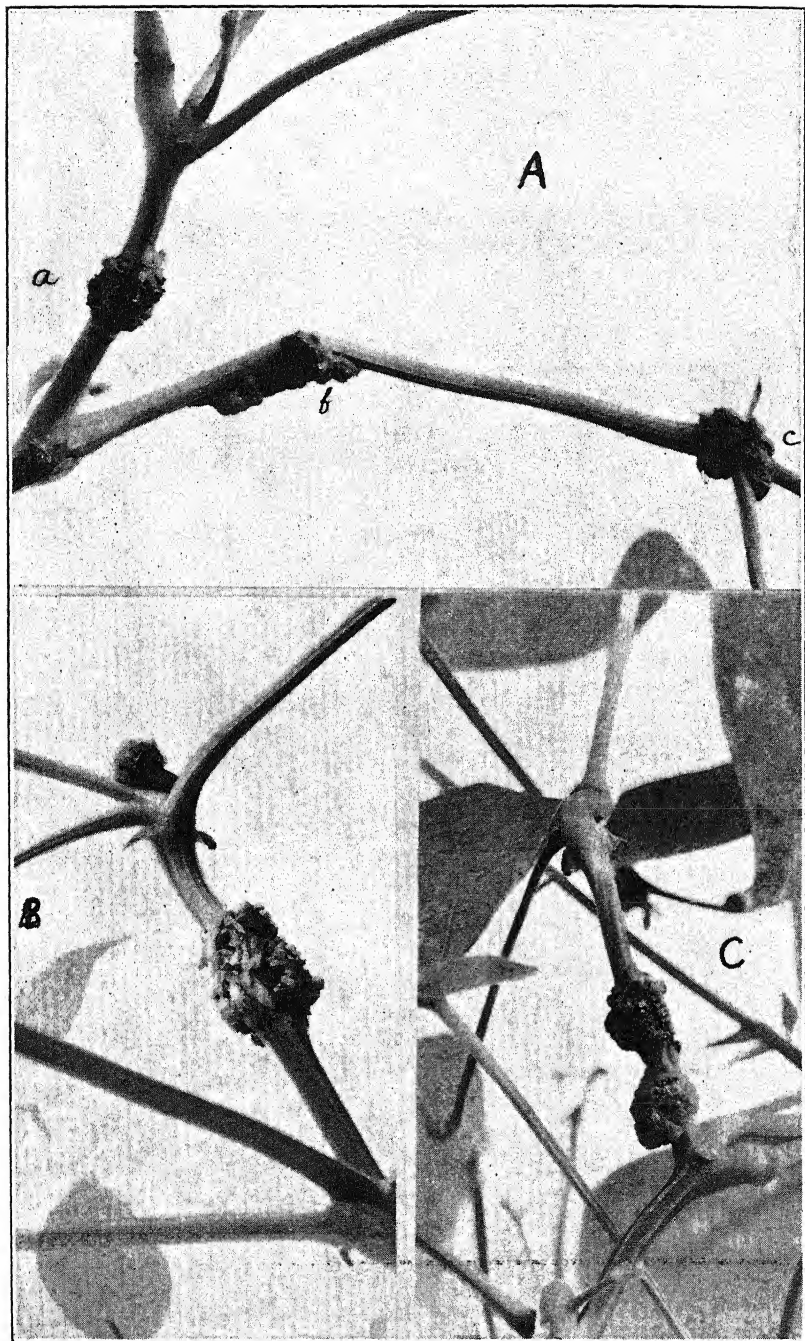


FIG. 2. Primary and secondary hormone galls on Kidney bean stems. A. Primary gall on bean stem produced at *a* by smearing indoleacetic acid-lanolin mixture on stem in a ring without wounding, 11-9-36. *b* and *c* are secondary galls formed later on the stem independently. Photo. 1-6-37. B. Secondary gall above primary. C. Secondary gall below primary. B and C were smeared with the indoleacetic-lanolin mixture at region of primary gall and 11-9-36.

Most of the workers on crown gall do not consider the disease to be systemic because of the failure to isolate *Bacterium tumefaciens* from tissue adjoining a gall or from secondary galls. It is now apparent that the presence of the organism is not essential to the formation of galls of the secondary type, the secondary overgrowth being formed by the gall-stimulating substance given off by the bacterial organism in the primary gall. The appearance of secondary galls on bean plants bearing primary galls induced by indoleacetic acid indicates that the stimulus of gall-inciting substances can move through the stem for considerable distances.

Whether there is any analogy between the relation of these secondary galls to their primary ones, and the relation of animal primary tumors to their secondary tumors, is an interesting speculation.

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### SCLEROTIUM BLIGHT OF WHEAT<sup>1</sup>

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(Accepted for publication Aug. 18, 1937)

Sclerotium blight killed 10 to 80 per cent of the wheat plants in many fields for 7 years in Gallatin County, Montana. Hence, this disease was studied in farmers' fields and in experimental plats. The infested fields were found within 2 miles west of the Bridger Mountain Range, at altitudes approximating 5000 feet.

A specimen of sclerotium blight was collected in 1907, presumably in Montana, according to Hungerford.<sup>2</sup> Young<sup>3, 4, 5</sup> and Young and Morris<sup>6</sup> described the wheat blight caused by *Sclerotium fulvum* Fr. Lindau<sup>7</sup> (p. 823) stated: "*Sclerotium fulvum* Fr. zu *Typhula graminum* Karst. gehörig." Tasugi<sup>8</sup> described basidiospores of *Typhula graminum* Karsten

<sup>1</sup> Contribution from Montana State College, Agricultural Experiment Station. Paper No. 91 Journal Series.

<sup>2</sup> Hungerford, C. W. A serious disease of wheat caused by *Sclerotium rhizodes* in Idaho. *Phytopath.* 13: 463-464. 1923.

<sup>3</sup> Young, P. A. Sclerotium blight (*Typhula graminum*). U. S. Dept. Agr., Bur. Plant Indus., Plant Disease Repr. 13: 70. 1929. [Mimeographed.]

<sup>4</sup> ———. *Sclerotium fulvum* on winter wheat in Montana. *Ibid.* 15: 52. 1931.

<sup>5</sup> ———. Sclerotium blight destroys winter wheat in Gallatin county, Montana. (Abstract) *Phytopath.* 24: 21. 1934.

<sup>6</sup> Young, P. A., and H. E. Morris. Plant diseases in Montana in 1928. U. S. Dept. Agr., Bur. Plant Indus., Plant Disease Repr. Sup. 69. 1929. [Mimeographed.]

<sup>7</sup> Lindau, G. *Fungi imperfecti: Hyphomycetes* (zweite Hälfte). . . . In L. Rabenhorst, *Kryptogamen-Flora von Deutschland, Oesterreich, und der Schweiz*. Aufl. 2, B. 1, Abt. 9. Eduard Kummer, Leipzig. 1910.

<sup>8</sup> Tasugi, H. On the physiology of *Typhula graminum* Karst. *Jour. Imp. Agr. Expt. Sta., Nisigahara, Tokyo* 2: 443-458. 1935. [In Japanese. English résumé, pp. 457-458.]



causing snow rot of cereals. Remsberg and Hungerford<sup>9</sup> described the sclerotium diseases of grains and grasses.

#### SYMPTOMS OF SCLEROTIUM BLIGHT OF WHEAT

Snow, melting from fields of wheat, *Triticum aestivum* L., revealed areas of wheat killed by sclerotium blight (Fig. 1, A, B). The killed wheat crowns and leaves were white, light gray, or tan, and some were moldy. Many of the diseased wheat leaves lay on the soil and remained normal in shape (Fig. 2, E). The affected plants showed numerous red, brown, or black sclerotia, 160 to 640  $\mu$  in diameter in the leaves and sheaths (Fig. 1, C, D; Fig. 2, B, C, E). Usually all parts of diseased plants were killed. However, only parts were killed in some plants (Fig. 1, D). Such injured plants produced no heads, or small, light-color heads with shriveled seeds. Plants of this kind showed internal browning of the nodes in 1931, but not in 1933.

#### ASSOCIATION OF SCLEROTIUM BLIGHT WITH SNOW

Sclerotium blight killed the wheat plants only while they were covered with snow. The disease was most destructive in the parts of fields covered with snow banks as late as March 15. No development of the blight was seen after the winter snow banks melted.

From 1928 to 1935, sclerotium blight killed at least 100 to 500 acres of wheat annually in Gallatin County, Montana, except in 1934, when no trace of the disease was found. The weather was unusually warm, with very little snow from January to April, 1934, which may explain the absence of the blight that year.

#### EXPERIMENTAL PLOTS OF WHEAT

Nearly 300 rows of 37 varieties of winter wheat were planted in an experimental plot on a farm in the fall of 1931, incident to a study of stinking smut and stem rust. The plot was nearly ruined for this purpose by a severe infestation of sclerotium blight, which was, therefore, studied instead.

The effect of date of seeding on severity of sclerotium blight was studied for 12 varieties of wheat in this plot. The blight killed 30 to 90 per cent of the wheat sown on August 31, and Sept. 5, 15, and 25, 1931. It killed 10 to 60 per cent of that sown on October 5; very little of that sown on Oct. 15; and none of that sown on the 31st. The wheat planted on Oct. 15 and 31 emerged in greater part the following April, matured in unfavorable weather, and produced a poor yield.

Many plots of 14 varieties of wheat sown on the college farm at Bozeman had large areas killed by sclerotium blight by April 11, 1931. From 1926 to 1935, the blight was destructive on this farm only in 1931 (Fig. 1, B).

<sup>9</sup> Remsberg, Ruth, and C. W. Hungerford. Certain sclerotium diseases of grains and grasses. *Phytopath.* 23: 863-874. 1933.



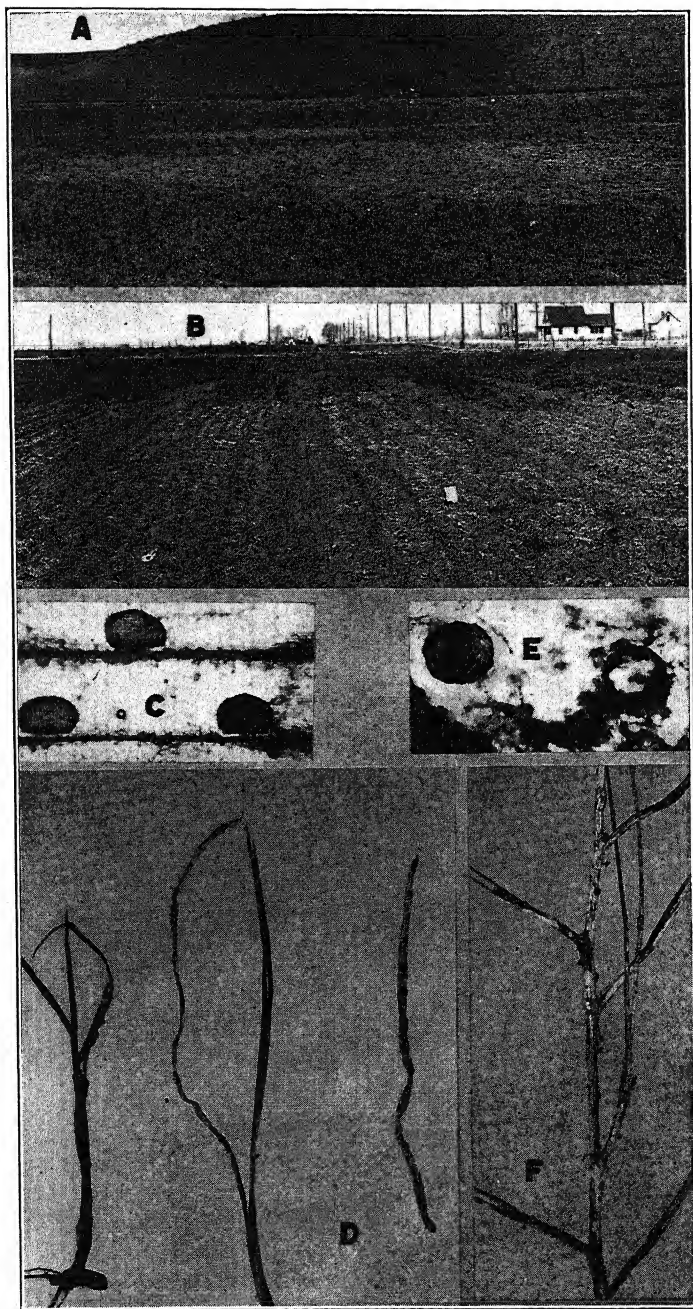


FIG. 1. A. Field of Jones Fife wheat showing areas killed by sclerotium blight in 1931. B. Experimental plots of Agronomy Dept., Montana Agricultural Experiment Station at Bozeman; foreground shows wheat killed by sclerotium blight, with living wheat in the background. C. Sclerotia of *S. fulvum* inside a sheath from an overwintered wheat stem.  $\times 7$ . D. Wheat leaves showing black sclerotia; the 2 wheat plants were alive but injured by sclerotium blight when photographed.  $\times \frac{1}{4}$ . E. Sclerotia of *S.*

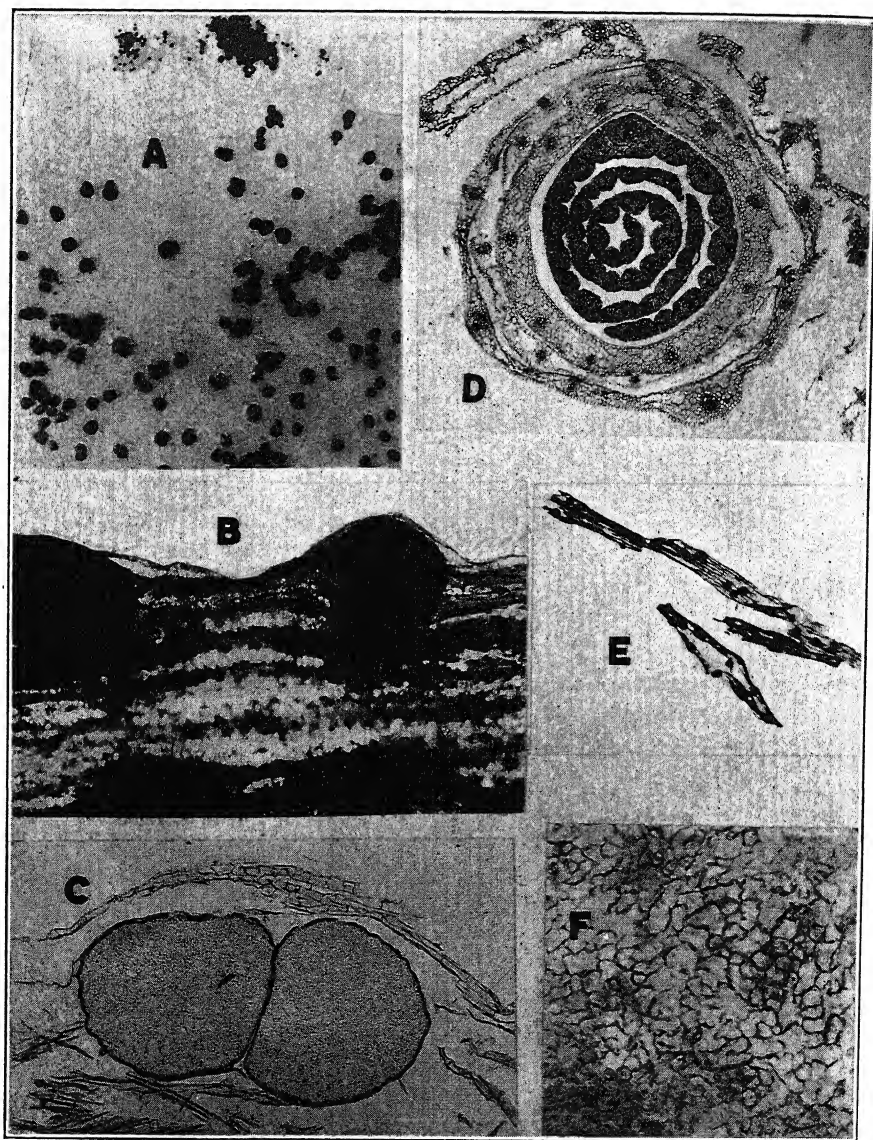


FIG. 2. A. Aggregate and single sclerotia of *S. fulvum* on agar; isolation from *Sisymbrium*.  $\times 8$ . B. Wheat epidermis naturally covering sclerotia of *S. fulvum* inside a wheat leaf.  $\times 26$ . C. Two sclerotia in a wheat leaf.  $\times 42$ . D. Cross section of a normal wheat bud showing structure of 2 cylindrically rolled leaves inside stem and sheath.  $\times 12$ . E. Light gray wheat leaves killed by *S. fulvum*, showing the black sclerotia.  $\times 1.6$ . F. Sclerotium of *S. fulvum* showing prosenchymatous surface structure.  $\times 241$ .

The following varieties of wheat were killed by sclerotium blight in 1931 and 1932: Albit, Blackhull, Cheyenne, Cooperaturka, Democrat, Fulcaster, Harvest Queen, Hussar, Hybrid 128, Jones Fife, Kanred, Karmont, Kawvale, Kharkof, Malakof, Marquis, Mediterranean, Minhardi, Minturki, Montana 36, Nebraska 60, Newturk, Oro, Regal, Ridit, Scotch Fife, Sherman, Tenmarq, and Turkey.

#### CULTURES OF SCLEROTIUM FULVUM

Sclerotia from overwintered stems of wheat and *Sisymbrium altissimum* were planted on corn-meal agar. Within a month, the sclerotia produced sparse, hyaline colonies that later bore numerous new sclerotia. They were white at first, but soon became yellow, orange, and red. They were reddish brown and 800 to 1200  $\mu$  in diameter, while turgid and mature. On dried agar, the sclerotia were black and 160 to 900  $\mu$  in diameter. The cultures from wheat produced, on agar, concentric rings of single sclerotia, while those from *Sisymbrium* produced many scattered, aggregate groups of 2 to 4 sclerotia (Fig. 2, A). Agar cultures of *Sclerotium fulvum* grew only at temperatures near 0°–5° C. This explains why sclerotium blight is closely associated with snow banks that provide the necessary temperature and moisture for the fungus. Sclerotia from wheat tissues and agar cultures were used in numerous experiments to try to induce formation of basidia, but none appeared. Hence, the name, *S. fulvum*, is retained here.

*Sclerotium fulvum* killed areas aggregating nearly 20 acres in a 100-acre field of Turkey wheat, observed on May 4, 1935. In a large area, where all of the wheat had been killed, all of the fanweed, *Thlaspi arvense* L., seedlings also were dead or dying from injury by *S. fulvum* (Fig. 1, E). The only normal green plants in this area were numerous seedlings of *Ellisia nyctelea* L., which thus appeared to be immune from *S. fulvum*.

#### ANATOMY OF SCLEROTIA

Sections of wheat leaves and stem sheaths showed that the sclerotia were inside the tissues. The sclerotia enlarged in the leaves and bulged the epidermis (Fig. 2, B, D). The sclerotia, black and wrinkled when dry, usually occurred singly, but sometimes in groups of 2 or 3 (Fig. 2, C). They were composed of modified mycelium forming prosenchymatous tissue (Fig. 2, F). Some of the inner tissue was pseudoparenchymatous. The sclerotia had an outer rind of 10 to 12  $\mu$  thick, with much thickened outer walls. The outer cells bulged outward, making numerous ridges on the sclerotia. In fields, decomposition of wheat tissues liberates the sclerotia into the soil where presumably they aestivate.

NATURAL SAPROPHYTIC DEVELOPMENT OF *SCLEROTIUM FULVUM*

A saprophytic stage of *Sclerotium fulvum* was discovered on overwintered wheat stubble and lodged straw as the snow was melting on April 23, 1932, and also in April, 1933. In an area where *S. fulvum* had killed much wheat in 1931, the overwintered straw bore, the following year, numerous sclerotia of this fungus on the culms and especially under the leaf sheaths (Fig. 1, C). Overwintered dead stems of *Sisymbrium altissimum* L. (Fig. 1, F) and *Chenopodium album* L., also, bore numerous sclerotia of *Sclerotium fulvum* in the above-described stubble field in April, 1932.<sup>10</sup> The sclerotia were found only on wheat straw and on stems of *Sisymbrium* and *Chenopodium* that had lain on ground where *S. fulvum* had killed wheat the preceding year. In such areas volunteer-wheat seedlings commonly were dead or dying with sclerotium blight. *S. fulvum* is a facultative parasite that increased abundantly as a saprophyte.

## SUMMARY

The principal symptoms and signs of sclerotium blight are: Killed leaves retain their normal shape as they lay prostrate on the soil; they are white, light gray, or tan, with numerous sclerotia visible in them; the sclerotia are 160 to 640  $\mu$  in diameter and black in dry leaves, but are much larger and yellow, orange, red, or brown in wet leaves; wheat plants, merely injured by sclerotium blight, produce small heads and shriveled grain.

In culture, *Sclerotium fulvum* grew only at temperatures near 0°–5° C, which explains why it is favored by temperatures near freezing under snow banks. Sclerotium blight was destructive only in those parts of fields covered by snow banks as late as March 15. Consequently, spring wheat escaped injury from the disease.

*Sclerotium fulvum* killed many plants of each of 29 varieties of wheat. This fungus also killed fanweed seedlings and produced sclerotia in them. The sclerotia of *S. fulvum* are produced inside wheat leaves. Natural decomposition of killed wheat leaves and crowns liberated the sclerotia into the soil.

*Sclerotium fulvum* produced saprophytically numerous sclerotia on overwintered stems of wheat, *Sisymbrium*, and *Chenopodium*.

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<sup>10</sup> See footnote 5.

## PHYTOPATHOLOGICAL NOTE

*Carbohydrate Utilization by the Corn Diplodias.*<sup>1</sup>—The unique character of the writer's recent findings regarding the sources from which a fungus now being studied obtains carbon in culture seems to warrant publication of this note. The fungus, *Diplodia macrospora* Earle, is one of the corn ear-rot fungi. In this study it has been compared with *Diplodia zeae* (Schw.) Lév., long recognized as an important cause of ear rot. This latter species apparently is found to some extent throughout the range of the host plant. On the other hand, *D. macrospora*, which differs from *D. zeae* chiefly in having much larger spores, has a rather limited distribution. At present *D. macrospora* is known only from the southeastern portion of the United States, Brazil, and Africa. Even in those localities in which both fungi are found, *D. zeae* is the more common.

That *Diplodia macrospora* grows much more slowly on culture media at room temperature than does the closely related *D. zeae*, was first reported by Miss Johann in 1935.<sup>2</sup> Stevens,<sup>3</sup> using a series of constant-temperature chambers at intervals of 5° C., found that this relation existed throughout the temperature range (approx. 15°–35° C.) of these fungi. The chief difference between the two is that the optimum for *D. zeae* is somewhat higher than for *D. macrospora*. Hoppe<sup>4</sup> found that the same relation held for the growth of these two fungi in ears of corn. Although *D. macrospora* invades ears readily when inoculated by itself, it is wholly unable to compete with *D. zeae*. When an ear of corn is inoculated with the two fungi, *D. zeae* alone is recovered. The writer has observed since that the more feeble growth of *D. macrospora* is also apparent when these fungi are grown on such media as potato-dextrose agar, corn-meal agar, bean-pod agar, and corn meal. From the studies reported in the present paper, it seems probable that the slow growth of *D. macrospora* may be associated with its inability to utilize the available carbon.

In the present study the growth of *Diplodia zeae* and *D. macrospora* is compared on a series of synthetic media offering different materials as sources for carbon. The media were made up on the basis of a modified Richard's solution as follows:

Distilled water, 1000 cc.; KNO<sub>3</sub>, 10 gm.; KH<sub>2</sub>PO<sub>4</sub>, 5 gm.; MgSO<sub>4</sub>, 2.5 gm.; source of carbon, a quantity sufficient to make an M/6 solution. In the case of starch and peptone a 10 per cent solution was used.

<sup>1</sup> The writer wishes to thank Drs. C. F. Hottes, N. E. Stevens, and O. E. May for helpful suggestions incident to the conduct of the research here reported, and Drs. H. J. Fuller and D. T. Engliss for materials not otherwise available.

<sup>2</sup> Johann, Helen. *Diplodia macrospora* on corn in Brazil. U. S. Dept. Agr. Bur. Plant Indus. Plant Disease Rptr. 19: 9–10. 1935. [Mimeographed.]

<sup>3</sup> Stevens, N. E. A note on the temperature relations of certain fungi. *Mycologia* 28: 510–513. 1936.

<sup>4</sup> Hoppe, P. E. Intraspecific and interspecific aversion in *Diplodia*. *Jour. Agr. Res.* [U. S.] 53: 671–680. 1936.

TABLE 1.—*Growth of Diplodia macrospora and D. zae on various synthetic media expressed in terms of average dry weight, in milligrams, of at least 6 cultures after 3 weeks' growth. In those two instances where growth is merely estimated, the cultures were too few to give reliable readings*

Carbon sources	<i>D. zae</i>	<i>D. macrospora</i>
Peptone .....	125	50
Starch .....	415	105
Lactose .....	212	100
Maltose .....	420	200
Sucrose .....	415	150
Sucrose and glucose .....	293	117
Glucose-Difeo .....	390	0
Glucose-Mallinckrodt .....	320	0
Glucose-Pfanstiehl .....	310	0
Cerulose .....	285	0
Fructose .....	291	0
Glucose and fructose .....	290	0
Galactose .....	180	0
Xylose .....	Good growth	0
Arabinose .....	Good growth	0

For each medium the salts were weighed, dissolved separately, and then mixed. Twenty-five cc. of medium were placed in 125 cc. Ehrlenmeyer flasks and sterilized in the Arnold steam sterilizer for an hour on each of 3 successive days. For inoculations, 4-day-old Petri-dish cultures of *D. zae* and 6-day-old cultures of *D. macrospora* were used. Circular discs were cut sterilely from the margins of these colonies and dropped into the flasks. Each fungus was inoculated into 18 flasks of each medium; the flasks were then allowed to incubate 3 weeks on the laboratory shelves at a temperature of approximately 25° C., near the optimum as indicated by Stevens; readings were then taken by filtering off the colonies onto filter paper that had previously been weighed, rinsing with distilled water, and drying in the oven at a temperature of 38° to 40° C. Finally, the discs with the dried fungal mats were weighed and the dry weight of the fungus calculated in milligrams.

The cultures of both fungi used in this work were from among those used by Stevens in his temperature studies. They were recovered from inoculated ears of corn at Arlington Farm, Virginia, in the summer of 1935. Hoppe reported the existence of distinct strains of *D. zae*, but in this work only one strain of each fungus has been used.—KATHERINE KINSEL, Department of Botany, University of Illinois, Urbana, Illinois.

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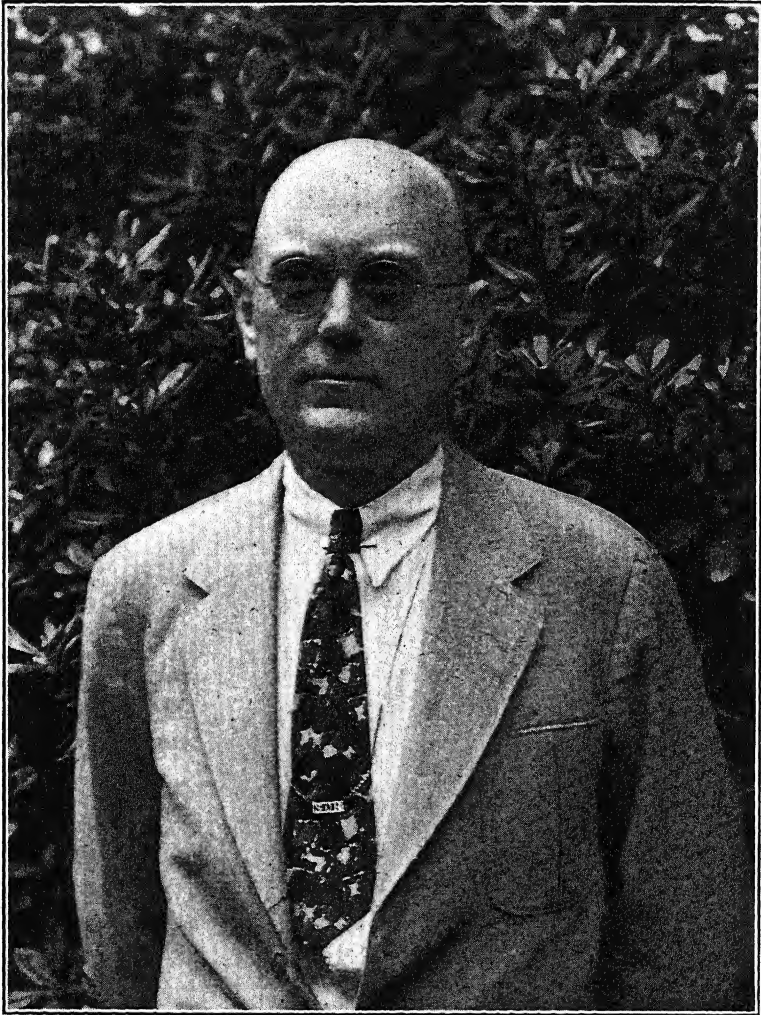
Abstracts should be submitted only by members who will present the papers in person. Not more than two papers under sole or senior authorship of one person will be accepted. Previously published material must not be included.

Authors should give (1) title of paper, (2) name and affiliation of author, (3) time required for presentation, (4) whether or not lantern is required.

**PUBLICATION OF ABSTRACTS.** The Society at the last annual meeting voted to publish in *PHYTOPATHOLOGY*, at the expense of the Society, abstracts of all papers presented at the annual meeting.

The Severin Hotel has been assigned as Society Headquarters, and the sessions of the Society will be held there, Monday, Dec. 27 to Thursday, Dec. 30, 1937.





STACY OTTO HAWKINS



STACY OTTO HAWKINS  
1899-1937

W. B. TISDALE

Stacy Otto Hawkins, Assistant Plant Pathologist in the Florida Agricultural Experiment Station, died at Gainesville, Florida, July 10, 1937, after a short illness.

He was born in Shelby County, Indiana, on September 6, 1899. His early education was received in the public schools of that county. After attending a summer session at Indiana University he taught for two years in the public schools of his home county. In the fall of 1919 he entered Indiana University and, after two years' study, he taught history and botany in Marion College, Marion, Indiana, for one year. He then returned to Indiana University and completed work in botany under Professor James M. Van Hook for the A.B. degree in 1923. After teaching a year in the Marion High School he again returned to the University for graduate work, but because of ill health he was unable to remain the entire year. However, he completed the work later and received the M.A. degree in 1926.

In December, 1925, Mr. Hawkins was appointed as Field Assistant in the Department of Plant Pathology of the Florida Agricultural Experiment Station and was located at Homestead, on the lower east coast, to conduct spraying experiments for the control of nailhead spot of tomatoes. The results of Hawkins' work during the following five years convinced the growers of the need for spraying vegetable crops to control parasitic diseases during the winter months. In 1931 he was promoted to Assistant Plant Pathologist and placed in charge of experiments for the control of phoma rot of tomatoes. During the following four years he developed successful means for controlling this disease. Early in 1936 he was transferred to Gainesville, where he was working on seed and soil treatments for the control of seed and soil-borne diseases.

Mr. Hawkins was a member of The American Phytopathological Society, the Indiana Academy of Science, and the Florida Academy of Sciences.

Mr. Hawkins was of a quiet and retiring disposition, although always cheerful and willing to assist his associates. He was an earnest and conscientious worker, and was always ready to encourage a worthy cause and help its advancement in any way he could.

Papers that bear Mr. Hawkins' name as author or co-author are:

Some Xylarias of Indiana. Indiana Acad. Sci. Proc. (1925) 35: 225-229. 1926.

Some effects of lightning on tomato plants. Indiana Acad. Sci. Proc. (1932) 42: 57-59. 1933.

Gray leafspot, a new disease of tomatoes. Florida Agr. Expt. Sta. Bull. 249. 1932.

Control of phoma rot of tomatoes. Florida Agr. Expt. Sta. Press Bull. 467. 1934.

Experiments for the control of phoma rot of tomatoes. Florida Agr. Expt. Sta. Bull. 308. 1937.

Tomato diseases in Florida in relation to cultural practices. (To be published as a Florida Station Bulletin.)

## THE PARASITISM OF POLYPORUS SCHWEINITZII ON SEEDLING PINUS STROBUS<sup>1</sup>

ROBERT E. WEAN<sup>2</sup>

(Accepted for publication August 24, 1937)

### INTRODUCTION

*Polyporus schweinitzii* Fries, as a cause of root rot in coniferous trees, was reported by Sargent (23), in 1897, to be more destructive in the United States than in Europe. Von Schrenk (25), in 1900, stated that this organism was very common in our northern forests of spruce and fir. In 1933, York<sup>3</sup> discovered *P. schweinitzii* causing root and root-crown decay in forest plantings of white pine, *Pinus strobus* L., established in 1912-1914.

It is generally believed that this fungus attacks mature and over-mature coniferous trees by invasion of the roots through wounds. The penetration of root parasites into woody plant tissue has been extensively studied by several investigators (6, 11, 18, 25, 29). However, our knowledge pertaining to *Polyporus schweinitzii* is based entirely upon field observations. From this information it appears to have a wide range of coniferous hosts (2, 8, 12, 13, 21, 25, 34). It also has been reported on hardwood trees (15, 22). The writer is not aware of any published record of the ability of this fungus to enter its host directly or of infecting and injuring the living roots of seedling conifers.

York's conclusion (*op. cit.*) that this organism is, apparently, a direct parasite and that there seems to be a close relation between the amount of infection and the pH of the soil suggested this investigation. The influence of temperature, hydrogen-ion concentration, and mineral nutrition on the

<sup>1</sup> A dissertation in botany, presented to the Faculty of the Graduate School, University of Pennsylvania, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

<sup>2</sup> The writer wishes to acknowledge the advice and criticism of Dr. H. H. York during the progress of the work and the facilitation of chemical analyses indicated. Appreciation is expressed to Mrs. Minnie Taylor York, Dr. Conway Zirkle, and Dr. W. G. Hutchinson for their suggestions regarding the manuscript.

<sup>3</sup> York, H. H. A study of a resinosis and root rot in forest plantings of *Pinus strobus* L. and *P. resinosa* Ait. [Manuscript.]

susceptibility of various plants to attack from fungous parasites has been demonstrated by numerous workers (3, 7, 9, 16, 27). Since *Polyporus schweinitzii* is now known to be exceedingly destructive in the Rochester watershed plantings of northern white pine in the State of New York, young trees of that species were utilized in this study in order to determine whether such factors as soil acidity and nutrition influenced the susceptibility of the host. A study of the physiology of the fungus also was attempted with a view to a more complete interpretation of host reaction.

#### MATERIALS AND METHODS

The original isolation of *Polyporus schweinitzii* used in this study was made from tissue of a sporophore collected from a severely infected white-pine plantation near Hemlock Lake in the state of New York. The inoculum was prepared by filling Petri plates two-thirds full of crushed white-pine needle duff to which was added sufficient 2 per cent Trommer's plain malt solution to secure a saturated condition. Having been autoclaved, they were inoculated at the center of the dish and held at room temperature in diffused light. The medium was ready for use when the fungus showed good growth over and through the duff.

The determination of the parasitism of young trees by this fungus involved the following procedure: 1. The use of seedlings immediately following germination of seed, and 2-year-old trees from a nursery. 2. Plants grown under 5 different nutritive conditions in the greenhouse, *i.e.*, a complete nutrient solution adjusted to the pH points of 4.5, 6.0, 7.0, and 2 conditions of nutrient deficiencies at pH 4.5. 3. For each of the 5 conditions 12 pots composed a series; 8 of these pots were inoculated with *Polyporus schweinitzii*, and 4 served as controls, the latter having been added non-inoculated duff. In such an arrangement there were 60 pots of young seedlings and 60 of 2-year-old trees, each nutrient series being thus run in duplicate with the exception of the difference in age of the trees. Pot cultures of quartz sand, to which the nutrient solutions were supplied by the drip method, were deemed advisable for this study. The sand, after being thoroughly washed in running water, was placed in 6-inch clay pots for the young seedlings and 8-inch pots for the 2-year-old trees.

The white-pine seed used in this study was collected in 1933 in the State of New York. Prior to being placed on sterile sand in large moist chambers for germination, they were scarified with sand and were then surface-sterilized with 1-1000 bichloride of mercury for 2 minutes, and washed twice in 2 washings of sterile distilled water. A half plate of the medium was placed at a depth of 1 inch below the surface of the sand before planting 12 germinated seed in each container.

The 2-year-old seedlings were obtained from the Saratoga Nursery of the New York State Conservation Department. They were "lifted" from

TABLE 1.—*Growth and infection data of young seedlings<sup>a</sup>*

Series	Nutrient	Initial pH of nutrient	Shoots			Roots			
			Needles		Stem		Per cent infected	Number of laterals	Tap root
			Length	pH of extract	Length	pH of extract			
A <sup>b</sup> .....	Complete	4.50	mm. 21.2	5.20	mm. 69.4	5.60	20	14.9	mm. 109.5
A-e .....	"	"	21.2	5.25	70.0	5.60		16.3	236
B .....	"	6.00	19.5	5.26	64.6	5.63	37	20.7	112.0
B-e .....	"	"	22.3	5.15	66.0	5.50		22.9	80.7
C .....	"	7.00	25.3	5.00	66.5	5.60	52	23.5	88.9
C-e .....	"	"	25.4	5.27	73.8	6.12		27.2	95.3
D .....	Low nitrogen	4.50	27.6	4.85	70.3	6.10	28	17.5	92.5
D-e .....	"	"	27.1	4.95	72.3	5.65		20.0	81.0
E .....	Low phosphorus	4.50	24.7	4.70	59.8	5.30	52	14.4	115.0
E-e .....	"	"	24.5	4.70	61.0	5.60		16.1	44.8
									208
									mm. 5.00
									236
									275
									204
									308
									400
									422
									537
									582
									520
									302
									184
									208
									mm. 5.00
									4.96
									4.91
									5.15
									5.37
									5.82
									5.20
									5.30
									5.00
									5.15

<sup>a</sup> Average of 10 plants.<sup>b</sup> Series A, B, C, D, and E—inoculated; A-e, etc.—controls, noninoculated.

the seed beds in the fall of 1934, shipped to the University of Pennsylvania, potted in soil, and held over the winter in a cool greenhouse. On March 1, 1935, just as new growth was beginning, the plants were carefully removed from the soil and all foreign material washed off of the roots. Two plants were placed in each 8-inch sand culture. In this case a Petri plate of the needle-duff medium was put directly in contact with the root systems when the plants were potted.

Since few facts were available on the nutrition of white pine in sand cultures, the search for a satisfactory nutrient constituted a problem in itself, for it was necessary to provide one whose acidity might be altered over a wide range. The basic nutrient decided upon, following a personal communication with Dr. J. W. Shive<sup>4</sup> consisted of the following:  $\text{Ca}(\text{NO}_3)_2$  5.0 cc.;  $\text{KH}_2\text{PO}_4$  2.5;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  2.5;  $(\text{NH}_4)_2\text{SO}_4$  1.5;  $\text{Fe}_2(\text{C}_4\text{H}_4\text{O}_6)_3$  1.0;  $\text{H}_2\text{O}$  1000.0.

The salts were made up as molar solutions with the exception of the ferric tartrate of 1 per cent concentration. A nutrient solution composed of the above elements was used for Series A, B, and C after adjustment to pH 4.5, 6.0, and 7.0, respectively, by the addition of 0.1 N NaOH (Table 1). Two series with a reduction in the amount of an essential element completed the arrangement; namely, Series D in which the  $\text{NO}_3$  ions were partially replaced by Cl ions through the reduction of  $\text{Ca}(\text{NO}_3)_2$  to 1.25 cc. and the addition of 3.75 cc. of  $\text{CaCl}_2$ ; Series E in which the  $\text{PO}_4$  ions were partially replaced by Cl ions through the reduction of  $\text{KH}_2\text{PO}_4$  to 0.625 cc. and the addition of 1.875 cc. of KCl. The two latter series were adjusted to pH 4.5.

The methods utilized, whereby the nutrient might drip continuously into the pot-cultures, were of two types. For the young-seedling experiment the technique of Shive and Stahl (28), as modified by Trelease and Thomson (30), was followed. Because of the time and labor of maintenance in an experiment of this size, a different system was devised for the series containing the older plants. This system, as previously described by the author (33), consists essentially of a central reservoir for each series, an automatic flow meter, and a main feed line with a capillary tube leading to each pot.

The plants were maintained in culture for 220 days. During this time, root isolations were made for the organism and data upon the plant reactions were obtained. At the end of the period, the plants were carefully lifted from the sand, washed, and the growth and infection data recorded.

The "typical plant," as outlined in Table 1, is a result of an average for 10 plants selected at random in the control and inoculated pots of each series. In the study of the 2-year-old plants (Table 2), 8 trees from the inoculated pots and 4 from the controls, selected as above, were carefully

<sup>4</sup> Shive, J. W. New Jersey Experiment Station, New Brunswick, New Jersey.

TABLE 2.—Data showing the average growth of the 2-year-old trees during the experiment<sup>a</sup>

Series	Nutrient	Initial pH of nutrient	Shoots			Roots	
			Needle length	Stem length	New growth	Number of roots	Total new root growth
			mm.	mm.	mm.		mm.
A <sup>b</sup> .....	Complete	4.50	60.0	151	166	265	2116
A-c .....	"	"	81.0	156	200	239	2264
B .....	"	6.00	68.3	109	142	200	1614
B-c .....	"	"	70.5	139	215	305	2497
C .....	"	7.00	74.3	106	106	374	2251
C-c .....	"	"	75.1	153	171	441	3746
D .....	Low	4.50	77.9	125	161	163	1915
D-c .....	nitrogen	"	80.0	140	183	218	2541
E .....	Low	4.50	65.6	148	155	161	2068
E-c .....	phosphorus	"	81.9	144	189	277	2692

<sup>a</sup> Based on 8 inoculated and 4 control plants for each series selected at random.

<sup>b</sup> Series A, B, C, D, and E—inoculated plants; A-c, etc.,—controls, noninoculated.

examined in each series. The extract for pH determinations was secured by grinding the plant tissue with damp quartz sand in a mortar. To the extract was added 10 cc. of distilled water prior to making the readings electrometrically with a quinhydrone electrode. Root material was fixed for histological study in a saturated aqueous solution of salicylic acid plus chromic sulphate and formaldehyde (4). Representative plants for each series were reserved for photographic purposes and ash analyses.

Since reddish areas have been reported occurring in roots infected by *Polyporus schweinitzii*, an injection experiment was made in an attempt to determine whether a fungus extract would produce a similar change in woody tissue of young white-pine trees. The extract was made by grinding fungus mats with sand in a mortar and filtering the liquid, plus distilled water, through a Seitz filter. This extract was introduced by capillary injection into the stems of 3-year-old trees. In similar manner sterile-needle punctures and injections of distilled water were made in control trees. After a period of 3 months, sections from plants thus treated were made for microscopic examination.

In order to determine whether during growth the fungus altered the pH of a medium, duplicate liquid cultures of surface and submerged type were arranged over a pH scale of 2.59 to 7.0. The nutrient consisted of a 2-per cent Trommer's plain malt solution adjusted to the various pH points by the addition of hydrochloric acid and sodium hydroxide.

For the purpose of determining whether an acid was produced by this fungus during growth, culture solutions, buffered by potassium hydrogen phosphate and citric acid, were arranged over a pH scale of 2.0 to 8.0. All cultures of the fungus were held at room temperature and secluded from

direct light. Examination for changes in the medium was made after 14 days.

#### RESULTS

*Pot Cultures of Young Seedlings.* The plants in the control cultures showed that distinctive growth reactions occurred when young white-pine seedlings were grown under various nutritional conditions. Physiologically, the only variables for the different series were introduced by varying the pH and the mineral content of the nutrient solutions. The character of growth response for each series and the results from inoculations with the fungus are shown in table 1.

The apparently normal seedling plants in the control cultures of Series A, receiving the full nutrient at pH 4.5, developed sturdy stems and bright green needles, and their light brown roots bore many root hairs (Fig. 1, A). The response of the inoculated plants in this series was not strikingly different from that of the controls. Root and shoot development was not seriously impaired, although 20 per cent of the lateral roots were infected. In a few cases (Fig. 1, A) the plants sustained severe root injury. The infection of the roots was characterized by localized brownish areas, and the portion toward the root tip ultimately died.

The control plants of Series B (Fig. 1, B), with the full nutrient adjusted to pH 6.0, were apparently less healthy than those of Series A. While the shoot growth was about equal, the needles were a paler green. The root system of the plants in Series B was more extensive and bore more laterals with well-developed root hairs. The inoculated seedlings of this series were more heavily infected than those of Series A, since infection occurred on 37 per cent of the roots. Root and shoot formation was noticeably less than that of the controls; death of the tap root was characteristic of these plants (Fig. 1, B).

The seedlings of Series C, grown at a pH of 7.0, differed decidedly from those of Series A and B (Fig. 1, C). The needles, although well developed, were a pronounced pale yellow-green. Although the stem growth was surprisingly vigorous, the root system was dark brown, and consisted of many fine, short laterals. Root hairs were short and sparse. The inoculated plants showed a higher degree of susceptibility, as infection occurred on 52 per cent of the roots. The roots were near the surface of the sand (Fig. 1, C), as in the controls, thus penetration of the root system was not noticeably altered. Although the number of lateral roots increased with the adjustment of the nutrient solution toward pH 7.0, the number of infected roots increased in still greater ratio.

With a reduced supply of available nitrate nitrogen, the seedlings of Series D (Fig. 1, D) appeared, during the first 4 weeks of growth, to develop in height at a greater rate than those of the other series. This agrees

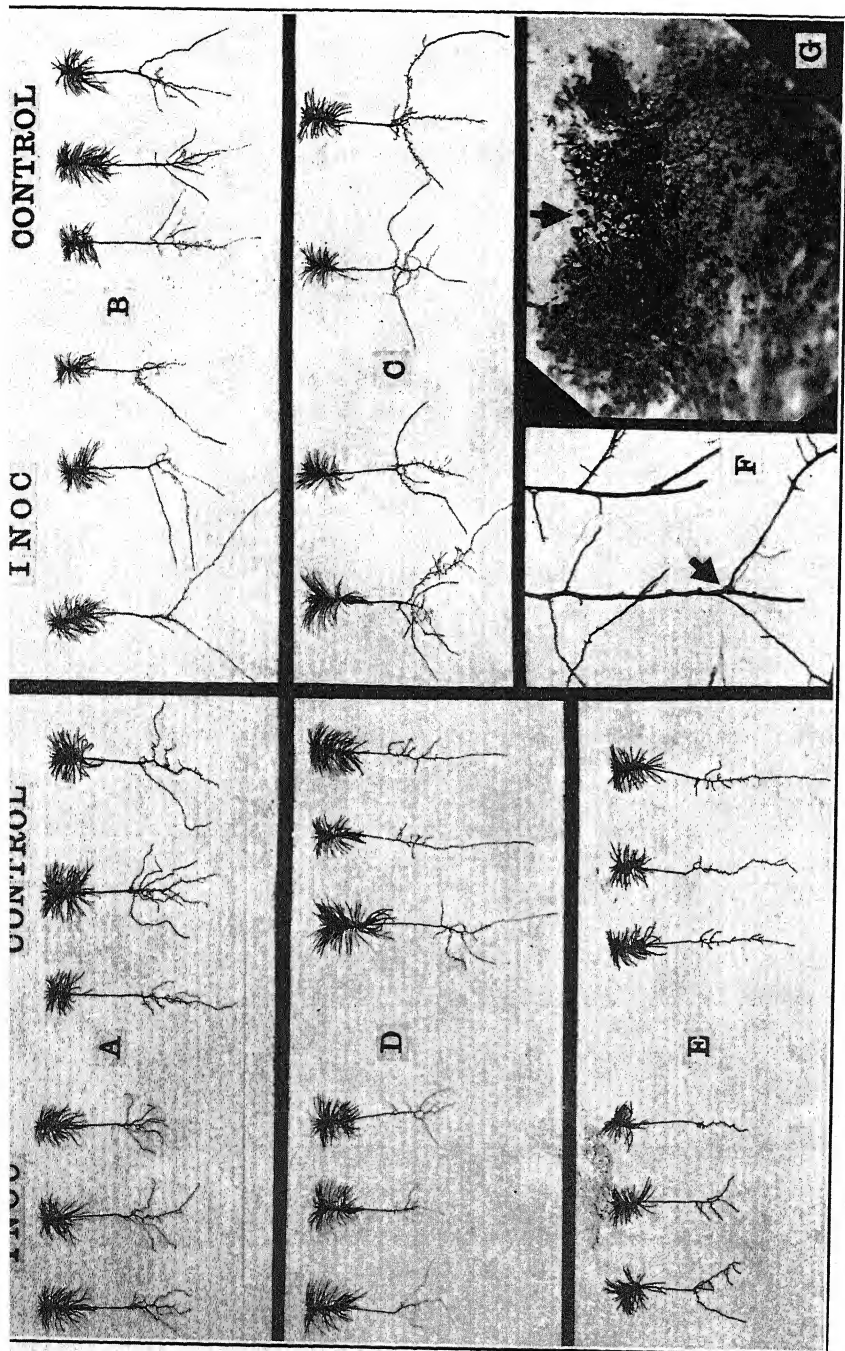


FIG. 1. Young seedling plants of control and inoculated cultures.  $\times 1$ . Series A, D, and E grown at pH 4.5; A, receiving the full nutrient; D, low nitrogen; and E, low phosphorus. Series B and C with plants receiving the full nutrient at pH 6.0 and 7.0, respectively. The control plants illustrate clearly the effect of changes in the nutrient solution on root development. Inoculated plants illustrate the typical taproot destruction on young plants, and the reduction in length and number of lateral roots by the parasitism of the fungus. F. Corky excrecences arising from the cork phellogen on the root systems, especially at the base of the lateral roots. G. Enlarged section of F showing the loose cell structure.  $\times 40$ .



with the growth of Corsican pine as recorded by Aldrich-Blake (1). This shoot reaction, however, was barely perceptible by the end of the experimental period. The diameter of the stems was slightly less than that of those receiving the full nutrient and the needles of a slightly paler green. The amount of root injury in the inoculated cultures, 28 per cent, was greater than under conditions of higher nitrogen concentration, and may be interpreted as showing that nitrogen deficiency may induce an increased susceptibility.

The reduced supply of phosphorus greatly retarded the growth of roots and shoots in the controls of Series E (Fig. 1, E). The needles, although of a deep green, were of normal length. Fifty-two per cent of the roots of inoculated plants were infected, which is identical with that occurring under conditions of a full nutrient at pH 7.0.

*Pot Cultures of 2-Year-Old Trees.* In many respects the older plants responded in a manner similar to that of the young seedlings in the different series. The control plants of Series A showed good shoot development, and the character of the root systems indicated favorable growing conditions. This was the only instance in which the number of new roots per plant in the inoculated cultures exceeded that of the controls (Table 2). From table 3 it is plainly evident that infection by the fungus was not severe on roots of the current season or on those of greater age.

The fact that the needles of the control plants at pH 6.0, Series B, were slow in assuming a normal green indicated less favorable growing conditions than those described above. Shoot growth was little affected except in color. There was an increase in number of roots and in the extension of the root system. Infection in this series was apparent on roots of both the current and previous seasons. Open lesions of decay were of common occurrence.

The poor color of new shoot growth at pH 7.0, Series C, was very marked; in fact, the color did not become a healthy green during the entire experimental period. The lateral roots were greatest in number and in total length of the 5 series, but they were very slender and a dark brown. The infection was severe (Fig. 2, C). The percentage of infected roots of the current season's growth was more than twice that of Series B and 5 times that of Series A. In the roots of previous seasons' growth, the percentage dead was nearly 3 times that of B and 6 times that of Series A (Table 3).

In Series D, at pH 4.5, low nitrogen appeared to be a factor affecting the growth of trees at this age, for shoot growth was less than that of Series A. The number of roots also was less, but the root system was of greater total length. The per cent of dead and infected roots of the current season and of the older roots was similar to that of Series B, but the development of lesions was less pronounced.

Under conditions of reduced phosphorus, shoot growth was again less than that of the full nutrient series at pH 4.5. The root systems were char-

TABLE 3.—*Root-decay analysis of 2-year-old trees<sup>a</sup>*

Series	Nutrient	Initial pH of nutrient	Roots of current season				Old roots			
			Roots	Dead	Infected	Lesions	Roots	Dead	Infected	Lesions
A inoculated	Complete	4.50	No. 265	Per cent 1.4	Per cent 2.4	No. 0.0	No. 354	Per cent 4.8	Per cent 4.2	No. 3.0
B "	"	6.00	200	5.5	5.5	4.5	275	11.0	9.8	4.5
C "	"	7.00	374	12.6	13.4	6.5	531	31.8	11.0	5.0
D "	Low nitrogen	4.50	163	3.0	6.0	1.0	334	16.0	8.6	1.5
E "	Low phosphorus	4.50	161	2.0	5.2	0.0	492	22.0	5.3	6.0

<sup>a</sup> Based on eight inoculated plants in each series selected at random.

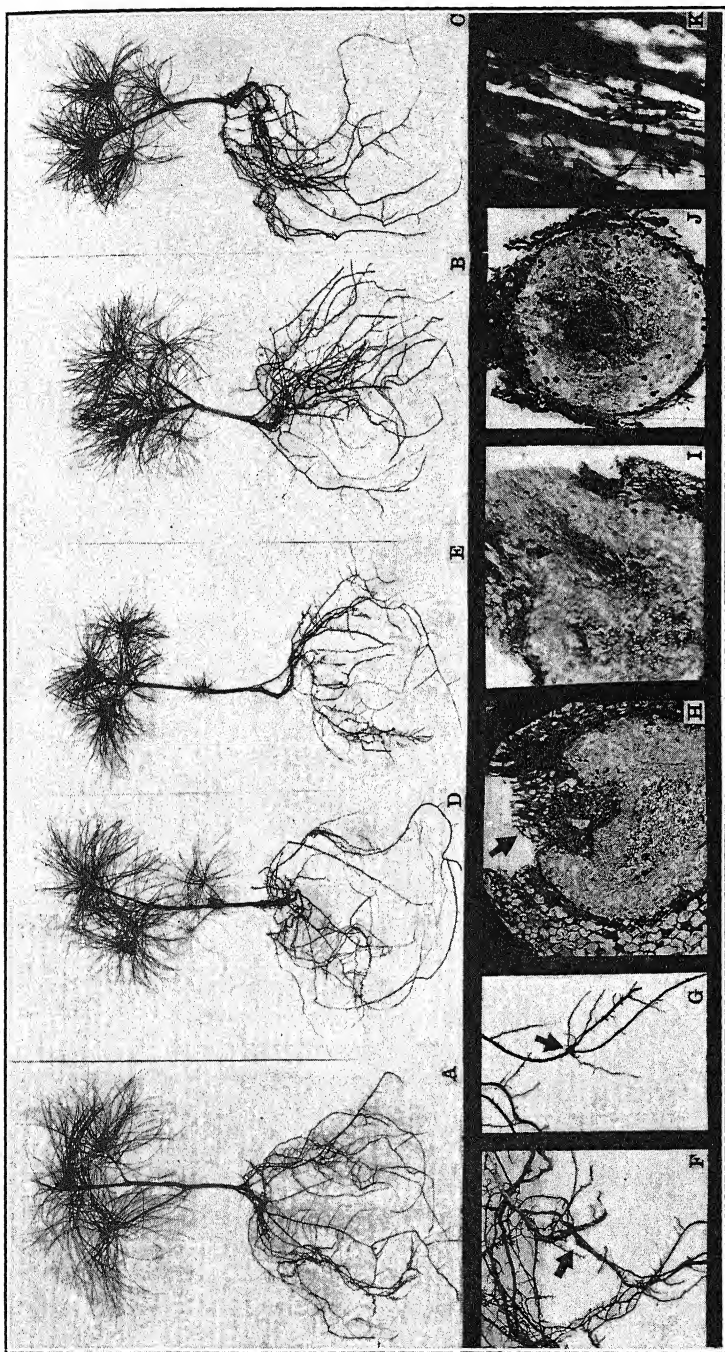


FIG. 2. Two-year-old trees from inoculated cultures.  $\times 1$ . Series A, D, and E grown at pH 4.5; A, receiving the full nutrient; D, low nitrogen; and E, low phosphorus. Series B and C with plants receiving the full nutrient at pH 6.0 and 7.0, respectively. Note the similarity of growth response in A and B, and the long, sparsely branched root system of D. The small root systems of E and C are the result of heavy infection by the organism. F. Enlarged view of lesions on the roots of the plant in Series C. G. Excessive branching of parasitized roots. H. Typical entrance of the fungus at the base of a parasitized lateral root.  $\times 40$ . I. Red discoloration in a lateral root extending toward the center of the mother root.  $\times 25$ . J. Transverse section of a root, the stained area in the center composed of heavily lignified cells.  $\times 25$ . K. The intracellular passage of *P. schweinitzii* within the tracheids of the parasitized root.  $\times 500$ .

acterized by short laterals of larger diameter than those of the other series. The percentage of infection on the roots of the current season was similar to that of the low nitrogen series, but that of the older roots was similar to that of Series C at pH 7.0 (Fig. 2, E).

It is significant that all inoculated cultures in the various series yielded plants with less new-shoot growth and smaller root systems than were found under similar conditions in the controls. Fewer lateral roots, likewise, developed in the former cultures, with the exception of the 2-year-old plants of Series A.

The results from pH determinations of juice extracted from the roots of both age classes of the plants showed a correlation with the shift in pH of the nutrient solution. Juices from the needles and stems, however, failed to show any substantial deviations.

Infected roots (Fig. 2, K) showed injury of various degrees of severity. In some instances the mother roots bore lesions (Fig. 2, F) with large open areas of decay. In others it appeared that certain root tissues had become meristematic in an attempt to wall out the fungus. Direct infection had often taken place through epidermal and cortical cells (Fig. 3, A and B), although the typical mode of entrance appeared to be either through a lateral or at its base (Fig. 2, H and I). Portions of roots killed by the fungus showed a dark red discoloration of the central region (Fig. 2, J) extending upward in the mother root toward the root-crown, occasionally even into the stems. The fungus could not be isolated from tissues very far distant from the point of initial infection, even though the discoloration in the central portion of the root extended beyond such areas.

*Chemical Analyses.*<sup>5</sup> York (*op. cit.*) found upon comparing the ash analyses of wood from normal white pine with that of trees infected with *Polyporus schweinitzii*, that the diseased trees contained less calcium than the healthy ones. The results from ash analyses<sup>6</sup> of plants in the inoculated and control cultures are shown in table 4.

The outstanding points in these results are the correlation in the absorption of calcium and phosphorus and the greatly reduced amount of calcium in plants grown at pH 7.0. This would appear to confirm the belief of Truog (32), namely, that the feeding power for phosphorus is related to the calcium content of the plant.

The fact that York (*op. cit.*) found, as a result of several hundred pH tests and analyses of water extracts of the soil, that there was on the average one-third more calcium in areas of diseased trees than in disease-free plantings suggested the possibility that *Polyporus schweinitzii* may inhibit the entrance of calcium into the roots of trees. Chemical analyses were made

<sup>5</sup> These data were loaned to the writer through the courtesy of H. H. York.

<sup>6</sup> The chemical analyses were made by H. J. Hallowell, Consulting Chemist, Philadelphia, Pa.

TABLE 4.—Ash analyses of seedlings and sand, indicated on a percentage basis of carbon and CO<sub>2</sub> free ash

Series	Seedlings							
	Total ash		SiO <sub>2</sub>		CaO		P <sub>2</sub> O <sub>5</sub>	
	1b	2	1	2	1	2	1	2
A <sup>a</sup> .....	3.89	3.99	2.48	7.90	8.72	12.93	23.75	21.49
A-c ...	4.02	3.50	2.29	7.89	10.08	11.05	23.76	28.75
B .....	3.94	3.03	2.39	7.07	10.02	12.02	23.51	21.93
B-c ...	3.74	3.31	3.14	6.82	10.63	16.80	25.87	27.05
C .....	4.20	3.33	3.77	8.68	6.23	8.35	18.35	21.99
C-c ...	5.32	2.81	4.20	6.23	6.72	13.88	21.35	24.75
D .....	4.06	2.93	1.60	5.25	9.86	7.68	25.38	22.02
D-c ...	4.48	3.22	1.51	6.80	10.34	10.84	28.63	25.82
E .....	4.14	2.87	2.66	8.68	7.93	7.59	22.85	20.02
E-c ...	4.61	3.32	2.22	9.40	10.80	15.40	26.76	25.28
Series	Sand							
B—Inoculated .....	0.054% CaO							
B—Control .....	0.0025% CaO							

<sup>a</sup> A, B, C, D, and E—Inoculated; A-c, etc.,—controls, uninoculated.<sup>b</sup> 1—Seedlings, 220 days old; 2—Two-year-old trees.

of water extracts for calcium in the quartz sand removed from the immediate vicinity of the roots of plants grown at pH 6.0. The results (Table 4) showed a percentage of only 0.0025 CaO in the control cultures, compared with 0.054 per cent about the roots of inoculated plants. Ordinarily, a root parasite is not considered as influencing the availability of nutrient material in the medium surrounding the roots of the host, but, under the conditions established in the sand cultures, it appears that *P. schweinitzii* caused the calcium to become less available to the plants.

*Histological Study.* Healthy and diseased roots were tested for cellulose and lignin content. For cellulose, potassium iodide with iodine and sulphuric acid were used, and for lignin, phloroglucin and hydrochloric acid. The infected roots showed a marked reduction in cellulose in the discolored areas, while the same zone evidenced heavy lignification of the cell walls. Compared with normal roots of similar age and size, the lignification of the woody elements was perceptibly advanced in the infected roots. It would thus appear that *Polyporus schweinitzii* produces an effect on the host that may hasten lignification of the cell walls (Fig. 3, C and D). Since the above tests gave similar results on tissue in which the hyphae were not present, but were discolored as a result of near-by infection, it seems that some product of the parasite was responsible for such host reaction.

When cross sections of diseased roots were stained with pH indicators, as that of the Hellige set and bromthymol blue of the Lamotte Chemical Company, the cell walls of the dead tissue acquired the color of the dyes in

their acid range, while the walls of the living, uninjured cells acquired the color of the dyes in their basic range. The acid color reaction also was found to occur upon treating normal white-pine wood known to be heavily lignified. These results indicate that the presence of *Polyporus schweinitzii* within host tissue causes a substantial change in the chemical constitution of the cell walls.

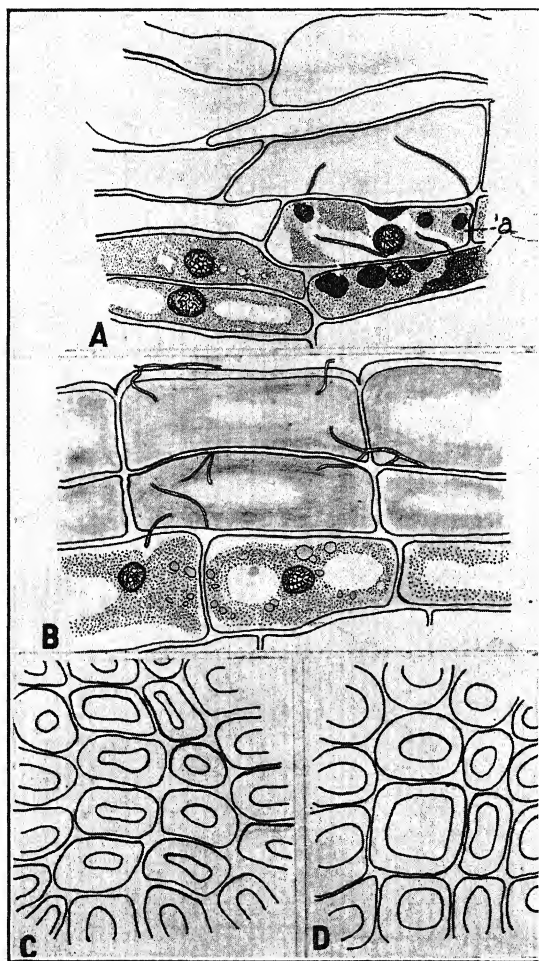


FIG. 3. Penetration of *Polyporus schweinitzii* into root tissue. Drawings made with the aid of a camera lucida.  $\times 650$ . A. Hyphae in the living cells of a young root. Note the break-down in cell contents of cells "a" in contrast with adjoining cones unaffected by the presence of the fungus. B. The direct penetration of hyphae through the heavy cortical layer of cells in a 2-year-old root. C. Heavily lignified cells from affected tissue illustrating the thick walls and small lumen of xylem cells. D. Structure of conducting tissue in the root of a control plant.

An examination of stem tissue near the root-crown, into which a fungus filtrate had been injected by means of a capillary tube, showed death of the cells about the point of entrance and reddish streaks in the surrounding wood cells. This was in contrast with the normal meristematic action of tissue about wounds made by the injection of water and needle punctures.

*Physiology of the Fungus.* A number of media proved satisfactory for the pure culture of this fungus. Growth typical of *Polyporus schweinitzii* occurred on oatmeal agar, Trommer's plain malt agar, whole buckwheat, white-pine-needle duff, and noncrushed white-pine seed. Little growth occurred on rice, and none on potassium silicate plus nutrients that had been found satisfactory for growth when added to agar medium. Trommer's plain-malt agar proved to be the most satisfactory for root-isolations and the storage of cultures.

Liquid cultures of 2 per cent Trommer's plain malt showed growth over the wide pH range of 2.59 to 7.0. After a 14-day period, however, the solutions made above pH 4.0 were found to be decidedly more acid than when inoculated (Table 5). The fungus assumed a green-yellow at points lower than pH 3.5 and a brown-yellow as the conditions were more alkaline. The effect of the fungus upon the solutions in surface and submerged cultures was not comparable in the more alkaline pH range.

TABLE 5.—*Liquid culture data of Polyporus schweinitzii* Fries.

Item compared	Initial pH	pH after 14 days	Diameter of surface growth in mm.	Character of growth	Culture acid equivalent after 14 days when buffered. CC. N/10 acid
Surface cultures	2.59	2.55	50	sb. <sup>a</sup>	
	3.20	3.20	70	sr.	
	4.00	3.60	82	sr.	
	4.65	4.00	90	sr.	
	5.18	3.65	85	sr.	
	6.00	3.62	65	sr.	
	7.00	4.20	40	sr.	
Submerged cultures	2.59	2.55	.....	sb.	
	3.20	3.21	.....	sb.	
	4.00	3.60	5	sr.	
	4.65	3.55	45	sr.	
	5.18	3.49	7	sr.	
	6.00	4.32	.....	sb.	
	7.00	5.00	.....	sb.	
Buffered solutions vol. 37.5 cc.	2.0	remained the same	45	sb.	5.25
	3.0		50	sb.	6.00
	4.0		60	sb.	6.00
	5.0		2	sb.	3.75
	6.0		1	sb.	1.50
	7.0		.....	.....	.....
	8.0		.....	.....	.....

<sup>a</sup> Sb.—submerged growth, Sr.—Surface.

Liquid cultures, buffered by potassium hydrogen phosphate and citric acid, showed a sharply defined pH optimum for this fungus (Table 5). Growth occurred at pH 2.0, but none above 6.0, while 3.0 to 4.0 appeared to be the optimum. The titratable acidity of the solutions was determined before inoculation, and 14 days later, using 0.1N NaOH, with phenolphthalein as an indicator. The amount of acid was found to be increased in all solutions where growth had occurred. From chemical analyses, it appeared that the increase in acidity was due to the production of succinic acid by the fungus.

#### DISCUSSION

An attempt has been made in this study to correlate the parasitism of seedling white-pine roots by *Polyporus schweinitzii* with growth conditions in sand cultures. For this study the 2 drip methods of supplying the nutrient, irrespective of the system, proved very satisfactory in maintaining growing conditions for the plants. The development of corky excrescences around points of emergence of laterals indicated that the sand had been kept well moistened (Fig. 1, F and G). Reactions to the modifications in the nutrient solutions were rather sharply defined, and it was quite evident that the growth response of young white pine can be determined best by a study of both shoot and root reaction.

There was a definite increase in the length of the root system and in the number of lateral roots as the nutrient solution was made more alkaline. However, with this increase in length and number of roots, the average root diameter became less, and thus the total root surface would not increase by so large a ratio as might seem to be the case. The development of the roots near the surface of the sand at pH 7.0 may have been due to the greater amount of phosphorus in that area. It is probable that a large percentage was precipitated into a less available form of ferric phosphate before penetrating far into the sand. Moreover, with the increase in alkalinity of the nutrient solution, the amount of calcium and phosphorus absorbed by the plants was decreased, and there was an increase in the alkalinity of the root extract. Since the susceptibility of white-pine roots to infection by this fungus appears in this experiment related to the chemical nature of the nutrient medium, the amount of calcium and phosphorus available must have an important bearing on host resistance. True (31) has contributed an explanation of the rôle played by calcium in the normal absorption of essential ions by plants, while Loew (17) has shown that phosphorus is of primary importance in the formation of new cells. At pH 7.0, the roots were typical of plants low in calcium (10), and reduced phosphorus proved to be a limiting factor to the increase in the size of seedling plants.

In the case of the older plants when grown at pH 4.5, a reduction in the amount of either phosphorus or nitrogen in the nutrient solution resulted in



greater extension of the root system. These results are in accordance with several previous studies (14, 19, 20, 24, 26). Low nitrogen in the case of the young seedlings produced a similar reaction, but low phosphorus unfavorably affected root length extension. The root systems of plants in the latter case were decidedly reduced in length, even though ash analyses showed phosphorus absorption to be nearly equal to that of the plants receiving the full nutrient at pH 4.5. It appears that the reduction in amount of available nitrogen and the increase in alkalinity of the nutrient solution affect the extension of the root systems of young white pines in similar manner.

The length of the shoots of the young seedlings was about equal, regardless of the acidity, when receiving the full nutrient. The older plants were more sensitive, however, with the least new shoot growth of the 5 series occurring at pH 7.0. Low nitrogen did not impair shoot development of the younger plants, although the stem diameter was less. A reduction of phosphorus suppressed shoot growth of young seedlings, as did nitrogen and phosphorus in the case of the older plants. From the above it appears that the effect of nutritional variations influences the development of roots more than that of the shoots of 1-year-old white-pine seedlings, while older plants may be expected to show rather sharply through their shoot development the influence of the medium in which they are grown.

The needles, regardless of the age of the plants, showed similar color reactions to the modifications in the nutrient solution. The needles of plants receiving the full nutrient at pH 4.5 were apparently normal in color. The reduction of phosphorus resulted in a deeper shade of green, but not a purple-green, as has been reported for phosphorus-starved plants. The yellow-green formed at pH 6.0 and 7.0 seemed to indicate low availability of iron.

The results of this investigation show that *Polyporus schweinitzii* is capable of parasitizing the roots of seedling white-pine trees under the conditions of this study. Parasitism occurred on both roots of the current and previous seasons' growth. Injury was evidenced by death of the small roots, the production of lesions, and red discoloration in larger roots. The presence of the fungus decidedly reduced the total length and number of lateral roots on the root systems of inoculated plants. Entrance into living host tissue was found to occur directly through epidermal and cortical cells, through the base of lateral roots, and through the corky excrescences at the base of some of the lateral roots. The intracellular passage of this fungus from one host cell to another occurred without evidence of mechanical pressure or constriction of the hyphae. Its entrance into the living roots of seedling plants indicates the possibility that this fungus may be distributed by nursery stock to forest plantings, and, furthermore, that it may be a factor affecting the survival of naturally reproduced white pine and possibly other conifers.

The seedlings grown under alkaline conditions and also those with low phosphorus and nitrogen were the most susceptible to attack. Hence, it appears that where these conditions exist singly or together, *Polyporus schweinitzii* may be expected to be especially destructive. The effect of this fungus upon absorption of mineral elements by its host is important, since the ash of infected plants showed a lower percentage of calcium than that of the controls, and an analysis of water extract of the sand from inoculated cultures showed a higher percentage of calcium than in the controls. Whether the calcium was made less available by the presence of the fungus, or whether the plants for some reason were unable to absorb the element, is problematical; the former interpretation seems the more logical, however, since it appears that the fungus produces succinic acid in liquid cultures.

Since an acid was produced by *Polyporus schweinitzii* during growth on buffered solutions, the red discoloration that occurred within infected roots may have been due to the acid liberated by the parasite within the host tissue. Furthermore, it seems that this excretion caused prematurity and death of the cells it penetrated. This may explain the observations on structural weakness of wood beyond the areas of visible decay (5). That this fungus produces a substance causing wood discoloration and change in cell character is further indicated by the fact that reddish streaks developed when a filtrate prepared from the mycelium was injected into woody tissue of 3-year-old white-pines.

#### SUMMARY

A physiological and pathological study of white-pine seedlings, inoculated with *Polyporus schweinitzii*, has been made in order to determine whether the fungus may become parasitic to living root tissue.

Root and shoot growth of the plants was found to be correlated with the pH of the nutrient solution and reductions of inorganic nitrogen and phosphorus. Low phosphorus proved to be a limiting factor in young seedling growth, while the reduction of nitrate nitrogen did not unfavorably affect it.

The absorption of calcium and phosphorus was reduced at pH 7.0 in both control and inoculated cultures. The presence of the fungus lowered the absorption of calcium by inoculated plants, as evidenced by the occurrence of relatively large amounts of calcium compounds in the immediate vicinity of the roots and a lower calcium content in the plant ash.

The hyphae of this fungus were found penetrating directly through living cortical cells of the root and corky excrescences at the base of lateral roots.

Parasitism increased with the alkalinity of the nutrient solution and with the reduction of phosphorus.

Infection of roots was accompanied by a reddening of host tissue. Such areas seemed to be prematurely lignified in young roots, and appeared to be

acid in character. The total length of the root system and the number of lateral roots were less in the case of infected plants.

The parasitism of living roots on plants of seedling age indicates the possibility of this fungus being spread by nursery stock to forest plantings and, further, indicates that it is a factor affecting the natural reproduction of susceptible host species.

*Polyporus schweinitzii*, in liquid cultures, produces an acid capable of increasing the acidity of nonbuffered solutions. On buffered solutions, the optimum point for growth is pH 4.0.

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# TWIG BLIGHT OF ASIATIC CHESTNUTS, ESPECIALLY THAT CAUSED BY PHOMOPSIS<sup>1</sup>

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## INTRODUCTION

The commercial stands of our native American chestnut (*Castanea dentata* (Marsh.) Bork.) have been almost destroyed by the ravages of the chestnut blight, an introduced disease caused by the fungus *Endothia parasitica* (Murr.) A. and A. Since there is very little, if any, prospect of obtaining resistant native stock, it seems desirable to consider other species that might be used to replace our valuable native chestnut for certain purposes.

In the consideration of replacement possibilities, attention is naturally directed to the Chinese species, *Castanea mollissima* Bl., and Japanese species, *C. crenata* Sieb. and Zucc. syn. *C. japonica* Bl., which have been grown successfully in orchards in many parts of the United States. These exotic species exhibit a high degree of resistance to the blight and possess some of the notably desirable properties and characteristics of our rapidly disappearing American chestnut. During 1930 and 1931 the United States Department of Agriculture distributed 188,223 Japanese and 14,550 Chinese chestnut seedlings throughout the entire eastern United States and these were set out in 162 separate experimental forest plantings to test the suitability of these trees for forest planting under our conditions of climate and soil. From 1930 to 1934, inclusive, the writer investigated the factors affecting these oriental chestnuts and with few exceptions every tree in these plantations was examined each year. The discussion here presented is based on 66,116 Japanese and 8,724 Chinese chestnut trees in 112 plantations distributed as follows: Alabama 2, Arkansas 5, Connecticut 4, Delaware 1, Florida 1, Georgia 3, Indiana 1, Iowa, 1, Kentucky 2, Maryland 6, Massachusetts 2, Mississippi 5, New Hampshire 2, New Jersey 13, New York 12, North Carolina 15, Pennsylvania 12, South Carolina 6, Tennessee 5, Texas 1, Virginia 10, and West Virginia 3.

The principal factors found affecting the trees in the experimental plantations have been listed in another publication.<sup>3</sup> The text of this paper will

<sup>1</sup> Many of the data herein presented were included in a dissertation submitted to the Faculty of the Graduate School of Yale University (Yale Forest School), June, 1932, in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Thanks are due to Professors J. S. Boyce and R. C. Hawley, of the Yale Forest School, for advice and encouragement. Acknowledgments are also made to several members of the Division of Forest Pathology for suggestions and assistance.

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<sup>3</sup> Bedwell, J. L. Factors affecting Asiatic chestnuts in forest plantations. Jour. Forestry 35: 258-262. 1937.

in part be confined to a discussion of twig blight with the range and damage caused to these hosts from 1930 to 1933, regardless of the fungi associated with the disease. There also will be given here some information concerning one particular twig-blighting fungus (*Phomopsis*) isolated in pure culture from infected chestnut twigs.

#### TWIG BLIGHT

When introduced into a different environment a plant is exposed to new hazards. It can be expected that many of our native twig and canker fungi of chestnuts and oaks, saprophytic as well as parasitic, might attack these introduced species of chestnut, especially if the trees or certain parts of them are in a weakened condition. Further studies are now under way by other members of this Division. In this paper the writer employs the term "twig blight" to include only fungus-induced dieback and cankers. Whenever the latter terms occur they apply only to dieback and canker caused by or associated with twig-blighting or cankering fungi.

#### FUNGI ASSOCIATED WITH TWIG BLIGHT

Twig blight of Asiatic chestnuts that have been planted in this country has been reported for several years, and the writer has examined specimens that have been sent to the Division of Forest Pathology and the Division of Mycology and Disease Survey from widely separated places. Several different fungi are found associated with the disease, some of which are probably saprophytic and not causal organisms producing a twig blight. On blighted Asiatic chestnut twigs the writer has found and identified fungi apparently native belonging to the genera *Phomopsis*, *Sphaeropsis*, *Diplodia*, *Cytospora*, *Diplodina*, *Macrophoma*, *Fusicoccum*, and *Phoma*.

#### PREDISPOSING CONDITIONS

Asiatic chestnut trees were found to be more subject to attack by twig-blight fungi when parts of the tree had been injured or killed by drought, low temperatures, mechanical injuries, browsing by grazing animals, or gnawing by rodents. Also, trees with lowered vitality, because of one or more of the above factors or from being planted on poor sites, were subject to serious injury by these organisms. In many cases it was difficult to determine the primary factor inducing dieback. Often, the killed portion of the twigs bore fruiting bodies of twig-blight fungi that might have been working as saprophytes; in many other instances the evidence indicated that killing was caused primarily by twig-blight fungi, although other factors might also have been involved. Injury in all such cases was considered to have resulted from the combined effects of twig-blight fungi and the other factors. It was quite apparent, however, that the twig-blight fungi were often able to become seriously parasitic, once the trees became infected. In compiling

and computing the field data a conscientious effort was made to classify properly the border-line cases and, considering the very large basis of plantations and trees, the writer firmly believes that the percentage figures are quite representative of conditions. In fact, the errors in diagnosis between the several factors involved are undoubtedly largely compensating.

#### DISTRIBUTION AND DAMAGE

Twig blight was found to be an important and serious factor affecting both Japanese and Chinese chestnuts over a wide range extending from New Hampshire to Georgia, west to Texas, and north as far as Iowa. This was particularly true on unthrifty trees growing on the poorer sites, or where predisposing conditions rendered the trees susceptible to attack by twig-blighting fungi. Extreme drought conditions proved injurious in 1930 and 1931 to recent forest plantings in Alabama, Delaware, Georgia, Maryland, southern New Jersey, North Carolina, South Carolina, Tennessee, Virginia, and West Virginia. At other places where chestnuts were planted, very little or no drought injury resulted to chestnuts or other species planted at the same time. In 1930 twig blight was of no consequence at plantations in the above-mentioned States or elsewhere, but in 1931, in most of these drought-affected States, it was significant. In a few of these States, however, there was only a comparatively small percentage of twig blight, *e.g.*, North Carolina, and, conversely, at plantings where drought was apparently not a factor on either the chestnuts or other species planted with them the incidence of twig blight was high (Cornwall Bridge, Connecticut, Amherst, Massachusetts, East Orange, New Jersey, Cornwall, New York, and Marienville, Pennsylvania). It has been suggested that there were possibly, at these places, late or early frosts that injured the twigs and furnished entrance points for the fungi. It is also a fact that some of these places were very poor sites, and at several of them rabbit and mouse injury was heavy.

For all locations as a whole, twig blight was serious in 73 per cent of the plantations in 1931 and had affected 52.6 per cent of the Chinese and 45.2 per cent of the Japanese trees. That year many plantations had from 90 to 100 per cent of trees affected and 13.1 per cent of the Chinese and 12.9 per cent of the Japanese chestnuts were killed by twig blight or by this factor in collaboration with one or more other factors.

Figure 1 summarizes graphically, by States, the percentages of trees killed by twig blight and other factors. For the living trees the graph shows the proportion affected by twig blight, and by other factors, and proportion healthy. In most of the plantings, as well as in most of the States as a whole, a higher percentage of the Japanese trees than of the Chinese trees were killed by twig blight. Of the trees living, however, a higher percentage of Chinese trees were affected.

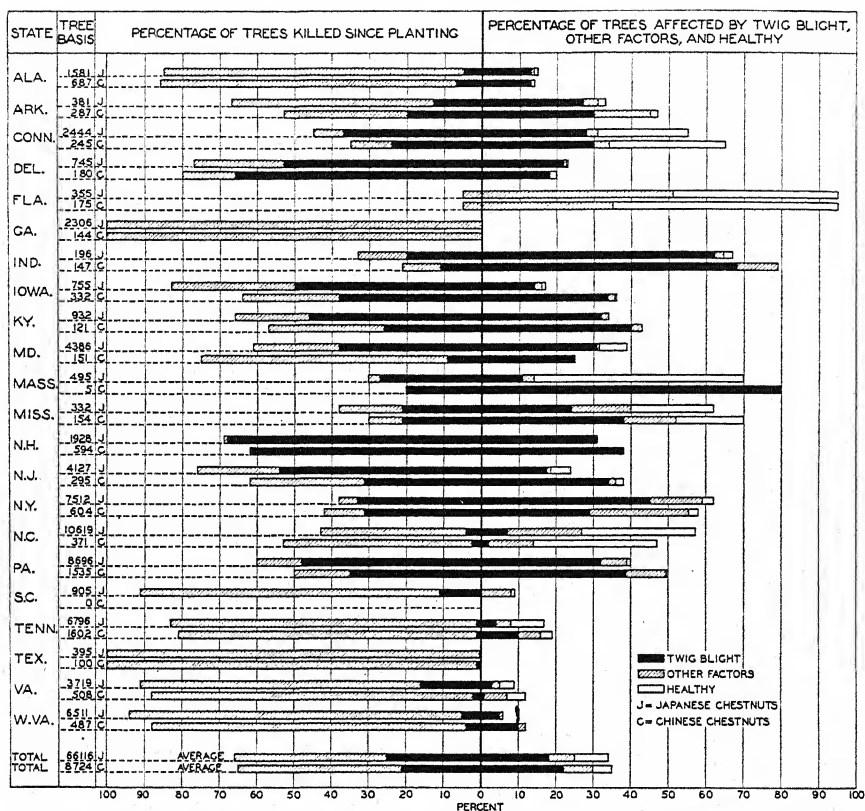


FIG. 1. Comparison of the distribution, by States, of twig blight and the damage caused to Asiatic chestnuts. The percentages of survival, living, and healthy, are figured on the tree basis listed.

#### PHOMOPSIS TWIG BLIGHT

The cultural and inoculation work done by the writer was confined to one of the organisms found associated with twig blight on Asiatic chestnuts in plantations in Arlington and Fairfax Counties, Virginia, and in Prince Georges County, Maryland. This fungus was determined as a *Phomopsis* and the writer believes it to be the same species that occurs commonly as a saprophyte on American chestnut and oaks.

#### SYMPTOMS<sup>4</sup>

The effect of the disease on the general appearance of the tree is noticeable at any time during the growing season, but it is most conspicuous during the spring months. Trees of any age or size may be attacked, but in the

<sup>4</sup> In general, the description hereunder also applies to twig blight, where fungi other than *Phomopsis* are associated with the disease.



larger trees usually only the small twigs and branches are affected. In many cases, attacked seedlings and saplings are killed outright. On the affected twigs the leaves wither suddenly without yellowing, gradually shrivel, and turn brown. This browning of the leaves and twigs gives the attacked part of the tree somewhat the appearance of having been scorched by fire. The fungus often stops at the point where the secondary shoot joins the main stem. These affected twigs sometimes rot at the base and fall off. On the diseased twigs are numerous small black pycnidia erumpent through the bark. These are sometimes arranged singly and sometimes in groups. When the fungus ceases growth at the intersection of the twig with the branch, only that type of the disease known as dieback results.

In many other cases the mycelium continues to grow on into the larger branches beyond the killed twig and develops the typical canker stage (Fig. 2). Likewise, infections through wounds on larger branches and limbs

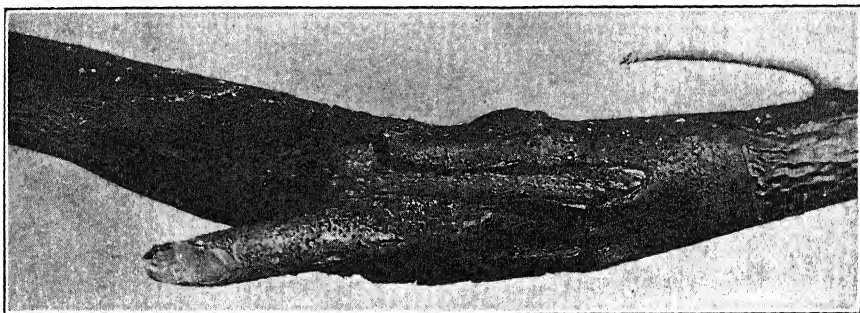


FIG. 2. Typical *Phomopsis* canker originating from twig-blight dieback through small twig. Two callus growths can be observed showing perennial nature of canker. Approximately natural size.

result directly in the typical canker stage. These cankered portions are slightly sunken and distinguished from the healthy light-brown or green bark by a dark-brown or blackish brown color. This demarcation between the diseased and healthy tissues is very sharp with a slight breaking away of the bark on the marginal portions. These cankers have been found on branches of all sizes up to 72 mm. in diameter. The usual shape of the canker is elliptical, being longer in the direction of the long axis of the branch. The margin of the canker is usually fairly regular, though not always so. In some cases it was found that cankers on the larger branches become deeper year by year as healthy tissues form a callus about them. This perennial canker, producing a distinct callus each year in a concentric pattern, accompanied by a falling off of the dead bark from the cankered area, causes a target-like condition somewhat similar in appearance to young *Nectria* and *Strumella* cankers.

As in the dieback form, the dead bark of the typical canker form is studded throughout its area with small black pycnidia. These fruiting pustules are at first covered by the periderm, which becomes lifted and finally ruptured, exposing the black stromata of the fungus.

Field studies showed that, in most cases, the typical *Phomopsis* canker results from the dieback form when the fungus grows through the twig into the limb. The majority of these cankers have a small dead twig in the center of the canker (Fig. 2). Field studies of the development of the canker also showed that the fungus started growth in the spring shortly before any external evidence of activity of the host was visible and reduced its rate of growth as the host resumed activity.

#### Artificial Inoculations

Inoculations were made in an effort to learn more about this development of cankers, whether or not the pathogen can attack healthy, nonwounded tissue and if it is parasitic on vigorously growing trees. Single-spore agar cultures of 2 strains of *Phomopsis* were used for the inoculum. One of these was from a twig-blight dieback specimen from Arlington Farm, Va., and the other was from a typical canker from Fairfax, Va. They were both from *Castanea crenata*.

Forty-eight healthy, vigorous, potted 2-year-old plants of Japanese and Chinese chestnuts, 24 of each species, were selected for the experiment. The 2 species were used to find whether any difference existed in their susceptibility to this twig-blight fungus. Plants in different stages of activity, from those completely dormant to those out in full leaf, were represented. This was done to find out, if possible, at what time the fungus made its growth in relation to the activity of its host. The experiment was conducted in the pathological greenhouses at Washington, D. C. Wound inoculations were made on 18 of the Chinese and on the same number of Japanese trees, the others being reserved as checks.

The places to be wounded were first cleaned by swabbing with 1:1000 mercuric chloride; then an incision was made with a sterile scalpel. Mycelium from the agar cultures was inserted in the wounds on some trees, while others received no inoculum and served as checks. The wounded places on the inoculated trees and checks were protected by wrapping with moist cotton and waxed paper. In other trees no incisions were made but inoculum was applied to unbroken tissue. In these tests the parts were washed and wrapped in exactly the same way as those that had been wounded.

Typical fungus-induced dieback and cankers bearing pycnidia resulted from these inoculations. In many cases the fungus progressed in the dieback form into the larger limb and formed a canker at the intersection of the twig and limb.

In these greenhouse tests the organism was very strongly parasitic, attacking vigorous trees growing under favorable conditions and killing twigs and branches and, in some cases, the entire tree. It is apparently a wound parasite, however, since infections occurred only in wounds and not in the wound-free or in the noninoculated checks. A few of the cankers were thrown off and the cankered area calloused over.

Trees in different stages of activity exhibited varying degrees of susceptibility to artificial infection. Fifty-three per cent of the wound inoculations were successful on trees dormant or just breaking dormancy, while only 34 per cent were successful on trees in full leaf. All of the trees killed were dormant or nearly so at the time of inoculation. The percentage of wounded and inoculated trees killed was the same (22 per cent) for both species and approximately the same percentage of infection was produced on both. The fungus was reisolated and both strains were grown in culture and produced pycnidia and spores of both the A and B type. These were identified as *Phomopsis* spores and the determination was verified by Glenn G. Hahn. Further inoculations from these reisolations resulted in typical dieback and cankers.

#### Development of Cankers under Natural Conditions

The canker appears at first as a more or less discolored area about the point of infection, which soon becomes sunken, making the boundary between the dead and living tissue very marked. The bark of such a diseased portion gradually dries up, and, in 4 to 8 weeks, produces numerous pustules, the initial stage of the stromata. A callus layer soon pushes out from the edge of the canker. The following year the fungus attacks this callus and an additional area of previously healthy bark, and, as before, a callus is formed around the newly killed area. Cankers have been found by the writer having as many as 4 of these concentric calluses. This gradual extension of the canker finally results in the girdling and death of the branch or bole.

Permanently tagged cankers and fungus-induced diebacks on 6 Japanese chestnut trees at Fairfax, Va., were examined and measured every 2 weeks from January 24, 1931, to May 16, 1931, inclusive. Another examination was made September 1 and a final examination January 19, 1932. It was observed that with most of the cankers and diebacks growth started about March 3 and stopped by May 1 and that the period of most rapid growth fell between March 3 and April 17. A few cankers showed a slight growth between the May 16 and September 1 examinations. No growth occurred on any of the infections between the examinations on September 1, 1931, and January 19, 1932. Pycnidia appeared on the surface of the newly cankered areas between April 2 and April 17 in every case but one. On April 17 the buds on the chestnuts were just beginning to break and on May 1 the trees

were out in full leaf. This harmonizes very well with the inoculation experiments where, it will be remembered, the best results were obtained with the dormant plants and the poorest with the plants in full leaf.

An example of what might happen under a certain set of conditions was furnished by a vigorously growing Japanese chestnut tree in the orchard at Fairfax, Va. On January 24, 1931, this tree was 9 feet tall and 2 inches in diameter at 6 inches above the ground and had 188 young twig-blight infections, or a little more than one for each of its 186 feet of live stem. All infections were carefully tagged and all the old killed parts of the tree were removed. On May 16, 1931, the tree was examined for new infections and 382 were found, which, together with those previously tagged, made a total of 570 infections on the tree up to that time. The twig blight had killed 63 linear feet of twigs and limbs between January 24 and May 16, 1931.

#### Dissemination of the Causal Fungus

Carriers of the disease are unknown. Under certain conditions of moisture and temperature, however, the pycnidia were found to extrude spore horns, consisting of a gelatinous mass containing conidia. This emphasizes the possibility of rain, insects, and birds as carriers. This twig-blight organism is quite similar in behavior, structure, and spore production to chestnut blight and other canker-producing fungi and it is a well known fact that birds, insects, and wind are the most common agencies in the dissemination of conidia of the chestnut-blight fungus.<sup>5,6,7</sup> Twig blight also might be locally disseminated by the use of cultivating tools and pruning shears, or for longer distances by shipping diseased nursery stock.

#### CONTROL OF TWIG BLIGHT

From observations and experience in connection with the chestnut nursery and orchard work, the writer believes it reasonable to conclude that practical control in forest plantations may be obtained by planting thrifty, disease-free stock on good sites where vigorous growth can be maintained, and the prevention of injuries as much as possible. Sites having good soil and air drainage should be selected and frost pockets should be especially avoided. In orchard plantings the same precautions should be observed, and, in addition, the trees should be cultivated and fertilized as necessary to keep them growing vigorously. Individual infections on valuable trees can be cut out, if the expense is justified. In any cutting or pruning opera-

<sup>5</sup> Carleton, M. A. The fight to save the chestnut trees. . . . Pennsylvania Chestnut Tree Blight Commission, Final Rept., pp. 25-60. 1914.

<sup>6</sup> Heald, F. D. Manual of plant diseases. 891 pp. McGraw-Hill Book Co., New York and London. 1926.

<sup>7</sup> ———, and R. A. Studhalter. Birds as carriers of the chestnut-blight fungus. Jour. Agr. Res. [U. S.] 2: 405-422. 1914.

tions, however, it should be borne in mind that wounds make excellent infection courts for these twig-blight organisms and other fungi.

#### SUMMARY

Twig blight of Asiatic chestnuts was found to be serious in young plantations, particularly on unthrifty trees growing on poor sites, on trees that had been injured or weakened by adverse climatic conditions, or on trees that had been wounded. Several genera of fungi were found associated with the disease, such as *Phomopsis*, *Sphaeropsis*, *Diplodia*, *Cytospora*, *Diplodina*, *Macrophoma*, *Fusicoccum*, *Dothiorella*, *Phoma*, and *Epicoccum*. In 1930 twig blight was of no significance, but, in 1931, it was serious in 73 per cent of the plantations and had infected 52.6 per cent of the Chinese and 45.2 per cent of the Japanese trees. That year 13.1 per cent of the Chinese and 12.9 per cent of the Japanese chestnuts were killed and many plantations had from 90 to 100 per cent of the trees infected.

Numerous controlled inoculations were made with cultures from a species of *Phomopsis* that had been found frequently associated with twig-blight cankers. The parasitism of this fungus on both Chinese and Japanese chestnuts growing in the greenhouse was proved by wound inoculations. Control plants with similar wounds remained healthy. Typical dieback and cankers bearing pycnidia resulted from these artificial inoculations. The fungus was reisolated and successful reinoculations were made with it. Susceptibility was highest in trees that were dormant or practically so and lowest in those that were out in full leaf.

The disease can be controlled if thrifty disease-free stock be planted on good sites, vigorous growth maintained, and injuries prevented.

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# A COMPARATIVE STUDY OF SOME EFFECTS OF SEVERAL DIFFERENT VIRUSES ON TURKISH TOBACCO PLANTS

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It has been shown that as much as 80 per cent of the total protein nitrogen readily extractable from Turkish tobacco plants, badly diseased with the ordinary strain of tobacco-mosaic virus, may be isolated in the form of crystalline tobacco-mosaic virus protein (15). The virus protein does not occur in normal plants (8, 12), and evidence now available indicates that it is the specific causative agent of the mosaic disease (14). That the production of large amounts of virus protein affects the metabolism of plants is indicated by the disease symptoms and by the fact that diseased plants contain larger amounts of total nitrogen and protein nitrogen than do normal plants (15). It has been known for a long time that the mosaic disease stunts Turkish tobacco plants, yet it is not known whether there is any correlation between the amount of virus protein in the plant and the severity of the stunting and other disease symptoms of the plant. Holmes (2), for example, has obtained a masked strain of tobacco-mosaic virus that has almost no visible effect on Turkish tobacco plants, yet causes the typical tobacco-mosaic, local-lesion response when inoculated to plants such as *Nicotiana glutinosa* L. His activity tests indicated that the masked strain of virus does not reach so high a concentration in Turkish tobacco plants as does the ordinary strain; hence it would follow that plants diseased with the masked strain should contain less virus protein than those diseased with the ordinary strain.

It also has been known for some time that the juices from Turkish tobacco plants diseased with different viruses, such as tobacco ring spot, latent mosaic of potato, and cucumber mosaic, would not withstand the dilution with retention of activity that the juice from Turkish tobacco plants diseased with tobacco-mosaic virus would withstand (11). This indicates that these viruses reach a lower concentration in Turkish tobacco plants than does tobacco-mosaic virus. The amount of tobacco-ring-spot virus protein in Turkish tobacco plants has already been determined and found to be about 0.01 mg. per gram of plant material (16), or about 1/300 the amount of virus protein usually found in mosaic-diseased Turkish tobacco plants. Because of the range of symptoms caused by tobacco-mosaic virus and its strains and by various other viruses, and because of the tremendous difference known to exist between the amounts of tobacco-mosaic and tobacco-ring-spot virus proteins in Turkish tobacco plants, it has seemed desirable to study the effect of ordinary tobacco-mosaic virus, some of its strains, and certain entirely dif-

ferent viruses on Turkish tobacco plants. This study has been carried out by determining the green weight, total nitrogen and protein nitrogen per cc. of extract, and the readily extractable protein of normal Turkish tobacco plants and of Turkish tobacco plants diseased with different viruses and grown under comparable conditions.

#### EXPERIMENTAL

The Turkish tobacco plants used in the present study were selected from a large group of young plants growing in 6-inch pots in a greenhouse. Nine sets of 9 plants each, comparable with respect to size and general appearance, were selected and placed in 9 rows across a greenhouse bench. When the plants were about 4 inches high, each of 8 of the sets was inoculated with a different virus. The inoculations were made by rubbing 2 leaves of each plant with bandage gauze pads saturated with the respective virus preparations. The remaining set of plants was allowed to remain healthy. The virus preparations used for inoculation consisted of solutions of crystalline tobacco-mosaic, aucuba-mosaic, and masked-tobacco-mosaic virus proteins containing  $10^{-3}$  gm. of protein per cc. in 0.1M phosphate buffer at pH 7 and the untreated juices pressed from Turkish tobacco plants diseased with latent mosaic of potato (X-virus), severe-etch, tobacco-ring-spot, green-cucumber-mosaic, and yellow-cucumber-mosaic viruses, respectively. The writer is indebted to Dr. W. C. Price for the last 3 viruses, and to Dr. L. O. Kunkel for the latent-mosaic and severe-etch viruses. At the end of 4 weeks the plants were cut just below the lowest inoculated leaf, weighed, and placed in a room held at  $-8^{\circ}$  C. When thoroughly frozen they were removed, put through a meat grinder, and 4 per cent by weight of disodium phosphate in the form of a 50 per cent aqueous solution was added. After the pulp had thawed thoroughly the juices were pressed out, filtered through thin layers of Hyflo Supercel, and their total nitrogen and protein nitrogen content determined. The total nitrogen and protein nitrogen were determined by Kjeldahl analysis, as previously described (5). The pulp from each set of plants was extracted with a volume of 0.1M phosphate buffer at pH 7 equal to that of the first extract. These second extracts also were filtered through thin layers of Hyflo Supercel and analyzed. The pulp cakes remaining after the second extraction were dried to constant weight at  $110^{\circ}$  C. and their total nitrogen content determined. The data obtained are presented in table 1. The experiment was repeated twice, once with plants inoculated when about 2 inches high and once with plants inoculated when about 6 inches high. Care was taken to work up the different sets of plants in as nearly comparable a manner as possible. Figure 1 is a graph presenting the data for the total weights of the freshly-cut plants in all 3 experiments. The data for the total nitrogen and protein nitrogen per cc. of the first extracts are given

TABLE 1.—Analytical data for medium-size Turkish tobacco plants after 4 weeks' infection with various viruses

Virus	Weight of 9 freshly cut plants	First extract			Second extract			Total protein extracted	Dry weight (110° C.) of residual pulp	Total N in residual pulp, N per gm. of pulp
		Volume	Total N	Protein N	Volume	Total N	Protein N			
	gm.	cc.	mg. per cc.	mg. per cc.	cc.	mg. per cc.	mg. per cc.	gm.	gm.	mg.
normal plants) .....	1910	1275	1.02	0.46	1205	0.32	0.07	4.02	151.0	76
red tobacco mo- saic .....	1582	955	0.82	0.50	925	0.25	0.12	3.52	113.0	25
in cucumber mo- saic .....	1460	980	0.85	0.36	875	0.21	0.08	2.52	52.8	44
ant mosaic .....	1500	910	0.85	0.36	910	0.25	0.12	2.63	85.3	20
acco ring spot .....	1367	920	0.95	0.47	910	0.27	0.10	3.14	71.0	80
are eteh .....	1063	740	1.10	0.47	715	0.35	0.14	2.69	50.5	43
ow cucumber mo- saic .....	1300	1015	(1.51)	(0.68)	1025	0.81	0.15	(5.07)	41.0	96
uba mosaic .....	1120	630	1.50	0.96	650	0.32	0.16	4.25	46.0	46
acco mosaic .....	1120	815	1.85	1.0	790	0.42	0.23	5.99	67.5	93



in figure 2. The total protein extracted from each group of plants has been calculated and is given in figure 3.

It may be seen from table 1 that there is considerable difference in the weights of the sets of plants diseased with different viruses and that in each case the weight is less than that of the normal plants. It also may be seen from table 1 that the volume of the juice or first extract was in each case approximately 70 per cent of the weight of the freshly-cut plants. The first extracts were found to contain about 4 or 5 times more total nitrogen and protein nitrogen than the corresponding second extracts. Subsequent extracts were found to contain but little protein nitrogen and to possess almost no virus activity. Therefore, over 95 per cent of the readily extractable protein was obtained in the first 2 extracts of frozen macerated plants. There was, however, a possibility that additional protein might remain in the extracted pulp. In order to determine the maximum amount of protein that might remain in the pulp, the twice-extracted press cakes were dried to constant weight at 110° C. and analyzed for total nitrogen. It may be seen from table 1 that the twice-extracted press cakes contained about 5 per cent solids, which in turn contained from about 2 to 10 per cent nitrogen. Despite the fact that the press cakes contained considerable nitrogen, it was not found possible to extract this nitrogen in the form of protein with either 0.1N or 1N sodium hydroxide or hydrochloric acid either at room temperature or at 100° C. The nitrogen remaining in the press cakes is apparently not in the form of soluble protein nitrogen. The data indicate, therefore, that from 80 to 90 per cent of the protein in frozen macerated tobacco plants is obtained in the first extract.

It may be seen from figure 1, in which the weight of each set of freshly cut plants is given, that every virus tested caused a stunting of the plants. Severe etch, when inoculated to small or medium-sized plants, caused severe symptoms and the most severe stunting of all viruses tested. When inoculated to large plants, however, it caused mild symptoms and but little stunting. With the exception of severe-etch virus, tobacco- and aucuba-mosaic viruses caused the most severe stunting. The masked strain of tobacco-mosaic virus, which caused almost no visible symptoms, had only a slight stunting effect on the plants. Latent-mosaic and cucumber-mosaic viruses, although causing more severe symptoms than the masked-tobacco-mosaic virus, nevertheless did not stunt the plants much more than the latter virus. Thus, although viruses producing the most severe necrosis and chlorosis usually cause the most severe stunting, such is not invariably the case.

The total nitrogen and protein nitrogen contents per cc. of first extracts from each of the sets of plants are given in figure 2. The tremendous increase in the protein nitrogen content associated with the tobacco-mosaic disease is most striking. In all 3 experiments the plants diseased with

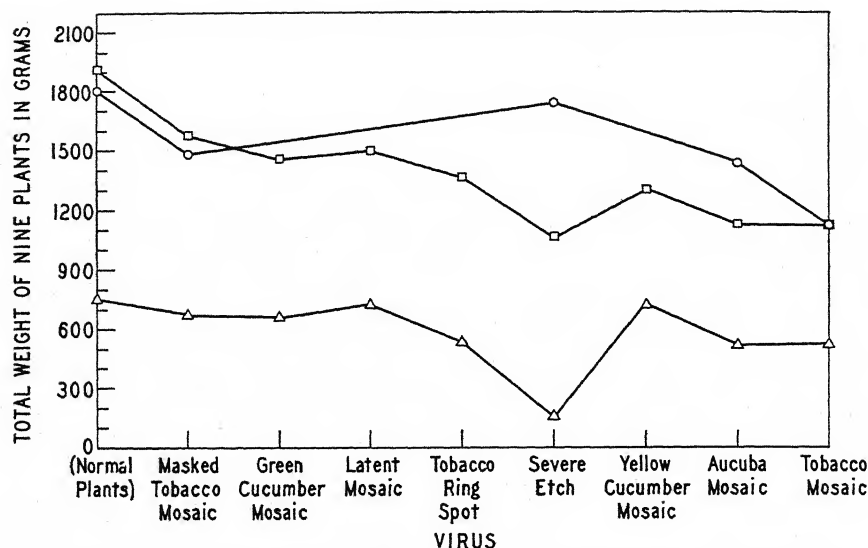


FIG. 1. Total weight of 9 freshly cut normal Turkish tobacco plants and of comparable sets of plants diseased with various viruses.  $\triangle$ , indicates plants were small;  $\square$ , medium-size; and  $\circ$ , large, respectively, at time of inoculation.

tobacco-mosaic virus contained between 2 and 3 times more protein nitrogen than the normal plants. Since it has been possible to isolate over 50 per cent

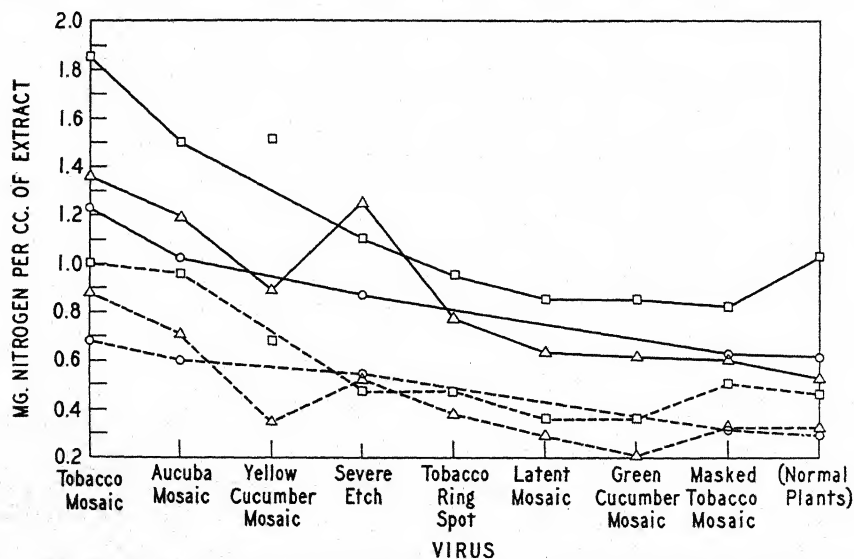


FIG. 2. Total nitrogen (solid lines) and protein nitrogen (broken lines) per cc. of the first extract from frozen macerated normal Turkish tobacco plants and from comparable plants diseased with various viruses.  $\triangle$ , indicates plants were small;  $\square$ , medium-size; and  $\circ$ , large, respectively, at time of inoculation.

and in some instances as much as 80 per cent of the protein nitrogen in the first extract in the form of crystalline tobacco-mosaic virus protein, it seems likely that the increase in protein nitrogen is due to the production of the high molecular weight virus protein. It has been found, for example, that mosaic-diseased Turkish tobacco plants contain a higher percentage of total nitrogen than mosaic-diseased tomato plants grown under comparable conditions, and that the extract from such tobacco plants is more infectious and contains a higher percentage of high molecular weight virus protein than does the extract from mosaic-diseased tomato plants (5). Aucuba-mosaic virus also causes a large increase in the total nitrogen and protein nitrogen content of the plant. The masked strain of tobacco-mosaic virus is quite different in its effect, for, in general, the total nitrogen and protein nitrogen contents were about the same as for normal plants. Recently several mild strains of tobacco-mosaic virus have been obtained and tested. In general, the mild strains produce symptoms and nitrogen contents intermediate between those of the masked strain and those of the ordinary strain. The total nitrogen and protein nitrogen contents given for yellow cucumber mosaic when inoculated to medium-size plants are probably abnormally high, for in the case of the small plants and several other separate sets of medium-size plants the values obtained were considerably lower and only slightly greater than those for green-cucumber-mosaic virus. Therefore, the values given for yellow-cucumber-mosaic virus in medium-size plants have been placed in parentheses in table 1 and, although designated in figures 2 and 3, have been disregarded in drawing the connecting lines. Tobacco-mosaic and aucuba-mosaic viruses appear to be quite different from all the other viruses tested in that they cause a marked increase in the total nitrogen and protein nitrogen content of the plants. Tobacco-ringspot virus and severe-etch virus usually cause a slight increase in the total nitrogen and protein nitrogen content, whereas the other viruses have no effect or a slightly depressing effect on the total nitrogen and protein nitrogen content.

Since tobacco mosaic and aucuba mosaic stunt the growth of plants, yet, at the same time, so stimulate the plants that the total nitrogen and protein nitrogen per cc. of extract is much greater than for normal plants, it seemed of interest to determine the net effect on the plant with respect to protein production. The total protein extracted from each set of 9 plants was calculated from the respective volumes and protein nitrogen concentrations of the 2 extracts. The results, which are given in figure 3, demonstrate that, although tobacco-mosaic and aucuba-mosaic viruses stunt the plant, they actually have a stimulating effect with respect to total protein produced. With the exception of severe etch inoculated to large plants, all the other viruses cause a decrease in the total protein produced by the plants. In general, therefore, tobacco- and aucuba-mosaic viruses are different in that

they actually stimulate protein metabolism. Although certain plant viruses are known to produce outgrowths (4) and certain animal viruses to cause marked cell proliferation (7, 9, 10), this is the first demonstration that a virus may act as a stimulatory agent with respect to protein production. The large amount of protein produced in tobacco- and aucuba-mosaic-diseased plants appears to be closely related to the intracellular crystalline deposits first noticed by Iwanowski (3) and recently well portrayed by Beale (1). This relationship is further emphasized by the fact that Beale found a general absence of intracellular crystalline material in Turkish tobacco plants diseased with the masked strain of virus. In the present study the masked strain was not found to cause the tremendous increase of

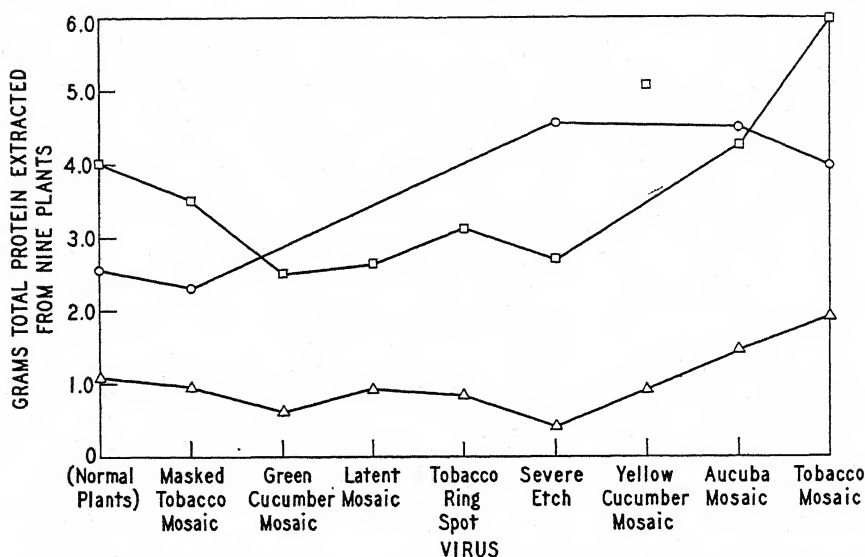


Fig. 3. Total protein (based on protein nitrogen  $\times 6$ ) extracted from 9 normal Turkish tobacco plants and from comparable sets of plants diseased with various viruses.  $\triangle$ , indicates plants were small;  $\square$ , medium-size; and  $\circ$ , large, respectively, at time of inoculation.

protein characteristic of the ordinary strain; hence, plants diseased with the former would not be expected to have the large deposits of crystalline material characteristic of the latter. The absence of intracellular crystalline deposits in plants diseased with latent-mosaic and tobacco-ring-spot viruses may likewise be correlated with the failure of these viruses to cause an increase in protein production. Beale's finding that aucuba mosaic causes larger amounts of intracellular crystalline deposits than does ordinary tobacco mosaic may be due to the lower solubility of aucuba-mosaic-virus protein (13), which would result in more protein in the form of crystalline deposits. Thus, although the total amount of protein in the cells might be

the same as or even slightly less than that in tobacco-mosaic-diseased plants, the amount of crystalline deposits might be greater because of the larger percentage of protein out of solution.

The results indicate that the large amounts of unusual high molecular weight proteins that occur in the case of disease caused by tobacco-mosaic virus and certain of its strains probably present an exceptional situation, since such stimulation of protein production was not found in the case of any of the other virus diseases studied. Recently it has been possible to examine, by means of an ultracentrifuge, the juices from plants diseased with various viruses. It was found that the juice from plants diseased with tobacco- or aucuba-mosaic virus contained about 3 mg. of high molecular weight protein per cc., whereas in the cases of the other virus diseases the juices were found to contain from about 0.001 to 0.1 mg. of high molecular weight protein per cc. (6, 16). This is additional evidence that tobacco-mosaic virus stimulates protein production much more than do the other viruses that have been studied and that this stimulation results largely in the production of high molecular weight virus protein.

#### SUMMARY

Tobacco-mosaic, aucuba-mosaic, masked-tobacco-mosaic, green- or yellow-cucumber-mosaic, severe-etch, tobacco-ring-spot, and latent-mosaic viruses, when inoculated to small, medium-size, or large Turkish tobacco plants, stunt the growth of the plants. Tobacco- and aucuba-mosaic viruses stimulate protein metabolism, however, so that, even though the growth of the plants is stunted, the total protein produced by the diseased plants is greater than that of normal plants. All of the other viruses studied caused a decrease in the total protein produced by the plants. Although severe symptoms and an increased protein content were characteristic of the tobacco- and aucuba-mosaic diseases, there appeared, in general, to be no direct correlation between the protein content of the diseased plants and the severity of the disease symptoms. The first extracts of frozen macerated plants were found to contain from 80 to 90 per cent of the extractable protein nitrogen in the plants. The extracts of plants diseased with tobacco- or aucuba-mosaic viruses were found to contain 2 or 3 times more protein nitrogen than the extracts of normal plants. This increase in protein nitrogen was found to be due to the production in diseased plants of large amounts of high molecular weight virus protein. The relationship between virus protein and intracellular crystalline deposits is discussed.

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# ISOLATION OF VARIANTS FROM CULTURES OF PHYTOMONAS STEWARTI

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## INTRODUCTION

Smith (15) concluded that *Phytomonas stewarti* (E.F.S.) Bergey *et al.* (*Bacterium stewarti* E.F.S.), the causal organism of bacterial wilt of corn, was a relatively stable species, since it retained its virulence and vitality on culture media for long periods. This conclusion has been commonly accepted, even though it is known that atypical strains may be isolated from infested soil (8) or infected plants (7, 12, 18). The stability of the bacterium in culture might be attributed either to the absence of variants or to the maintenance of a constant ratio between the strains that might coexist in culture. In order to determine whether strains were present, cultures were examined for variants of different pathogenicities. This was done by pouring dilution plates from pure cultures and testing the virulence of the single-colony isolates on sweet-corn seedlings. The observed differences in virulence of the isolates are reported in this paper.

## MATERIALS AND METHODS

Three cultures possessing the morphological and physiological characters described for *Phytomonas stewarti* by Elliott (5) were used. The culture (B-11), used in most of the experiments, was isolated from a sweet-corn plant of the variety Golden Bantam at Princeton, New Jersey, in September, 1935. The other 2 cultures had been grown on nutrient agar slants since they were isolated in New York and western Iowa in 1932 and 1933, respectively. In order to be sure of the purity of these 3 cultures, each was run through a series of 5 dilutions and single-colony isolations before it was used in the tests reported below. The single-colony isolate from the 5th dilution, which was used as the stock culture, produced typical wilt symptoms when inoculated into sweet-corn seedlings.

Nutrient broth (Difco) supplemented with 0.5 per cent dextrose and adjusted to a pH of 6.8 to 7.0 was employed as the nutrient base throughout the experiments. Single-colony isolates were obtained as follows from the stock cultures that were maintained on agar slants. Tubes containing broth were inoculated from the stock cultures, incubated for about 7 hours, and then thoroughly agitated before small samples from each tube were dispensed in melted agar for dilution plates. After 72 hours, single colonies were transferred to agar slants from a plate that contained less than 100 colonies.

Later, the isolates were transferred to nutrient broth and incubated for at least 72 hours before they were inoculated into test plants.

The plants used were sweet corn of the variety Golden Bantam grown in 4-inch pots filled with composted soil. Five seeds were planted in each pot and the seedlings were rogued to 3 or 4 plants before they were inoculated. All the plants for any one test were grown in the same greenhouse. Ten days after planting, 9 to 15 plants were inoculated with each isolate by injecting a small quantity of inoculum from a broth culture into the crown and leaf whorl of each plant by means of a hypodermic syringe. About 10 days after inoculation, the number of water-soaked and necrotic lesions formed along each invaded vascular bundle of the leaves, the number of dead leaves, and the total number of leaves on each plant were counted. From these data, an infection index for each isolate was computed as follows:

$$\text{Index} = \frac{\text{No. of lesions} + 3 \text{ times the no. of dead leaves}}{\text{Total no. of leaves}}$$

The dead leaves were arbitrarily classified as having 3 lesions each, since it was impossible to determine the number of lesions on leaves that underwent a rapid diffuse wilting or were killed by fusion of lesions. The infection indexes proved to be a convenient method of indicating the virulence of different isolates, since it was based upon the ability of the bacteria to cause wilting, as well as ability to invade the leaf veins sufficiently to cause a visible lesion.

#### EXPERIMENTAL RESULTS

##### Pathogenicity of Single-colony Isolates from Culture B-11

Twenty-four single-colony isolates were transferred to agar slants from a dilution plate of culture B-11 on July 4, 1936. Each of these isolates and the parent culture were tested for virulence in 3 different experiments. The infection indexes for each isolate in the 3 experiments are presented in table 1. Subcultures from the original agar slants were inoculated into 10-day-old plants (Experiment A) on July 17. The isolates were transferred to fresh agar slants in September. Duplicate subcultures seeded from the new slants were inoculated into 12-day-old plants (Experiment B) and 10-day-old plants (Experiment C) on September 14. In all 3 experiments the inoculated plants were examined for infection 8 days after inoculation.

Most of the isolates were similar to the parent culture, which had an infection index of 0.80 under the conditions of these experiments. However, some of the isolates were distinctly less virulent and a few appeared to be more virulent than the parent culture. On the basis of their infection indexes, the isolates were grouped into 4 classes: (1) slightly virulent, (2) weakly virulent, (3) virulent, and (4) highly virulent. The classes differed from one another not only in regard to their infection indexes, but also in the type of symptoms produced.



TABLE 1.—Average number of necrotic lesions produced on sweet-corn plants of the variety Golden Bantam by 24 single-colony isolates taken from infection plate of a pure culture of *Phytophthora stewartii* (B-11)

Isolate inoculated	Experiment A			Experiment B			Experiment C			Infection index <sup>a</sup>
	Total no. leaves	No. of lesions	Infection index	Total no. leaves	No. of lesions	Infection index	Total no. leaves	No. of lesions	Infection index	
ent culture	85	67	0.79 ± .08 <sup>b</sup>	49	37	0.75 ± .10	50	43	0.86 ± .11	.80
-11	80	0	0.00 ± .00	45	0	0.00 ± .00	61	0	0.00 ± .00	.00
ly virulent	91	18	0.20 ± .07	54	18	0.33 ± .09	53	10	0.19 ± .08	.23
ly virulent	80	58	0.72 ± .12	34	8	0.24 ± .14	57	18	0.32 ± .13	.49
11-165	77	42	0.55 ± .08	48	30	0.62 ± .09	52	32	0.62 ± .15	.59
11-172	65	35	0.54 ± .09	39	22	0.56 ± .13	48	34	0.71 ± .12	.60
11-161	92	54	0.59 ± .11	43	31	0.72 ± .19	39	31	0.79 ± .11	.67
11-163	77	63	0.82 ± .12	52	26	0.50 ± .07	39	26	0.67 ± .11	.68
11-158	86	59	0.69 ± .09	.....	.....	.....	.....	.....	.....	.69
11-164	78	59	0.76 ± .10	.....	.....	.....	.....	.....	.....	.76
11-173	85	71	0.84 ± .10	55	31	0.56 ± .09	48	36	0.75 ± .14	.73
11-156	80	53	0.66 ± .09	38	35	0.92 ± .13	49	36	0.73 ± .10	.74
11-159	84	66	0.79 ± .10	44	33	0.75 ± .14	49	34	0.69 ± .14	.75
11-171	80	68	0.85 ± .10	44	29	0.66 ± .12	39	26	0.67 ± .19	.75
11-168	87	71	0.82 ± .07	42	33	0.79 ± .15	51	29	0.57 ± .14	.74
11-170	89	68	0.76 ± .11	43	30	0.70 ± .12	51	34	0.67 ± .16	.73
11-175	76	64	0.84 ± .12	36	25	0.69 ± .15	29	16	0.55 ± .10	.74
11-174	84	63	0.75 ± .12	47	38	0.80 ± .19	38	36	0.95 ± .12	.81
11-169	85	67	0.79 ± .12	44	26	0.59 ± .13	40	46	1.15 ± .14	.82
11-167	84	71	0.85 ± .08	55	41	0.75 ± .12	36	33	0.92 ± .20	.83
11-178	91	81	0.89 ± .10	50	53	1.06 ± .18	35	27	0.77 ± .12	.91
ighly virulent	86	78	0.91 ± .11	60	56	0.93 ± .10	40	40	1.00 ± .15	.94
11-157	74	69	0.93 ± .06	45	38	0.84 ± .14	40	44	1.10 ± .17	.95
11-166	84	79	0.94 ± .13	52	54	1.04 ± .16	45	48	1.07 ± .21	1.00
11-160	78	58	0.74 ± .10	44	51	1.16 ± .14	43	57	1.33 ± .15	1.01
11-155										
11-176										

<sup>a</sup> Weighted average of 3 experiments derived by dividing the total no. of lesions by the total no. of leaves.

<sup>b</sup> Standard deviation of the mean.

The slightly virulent class included isolates, such as No. 163, which have an infection index of less than 0.20. The only macroscopic symptoms produced by isolates of this class were faint chlorotic streaks and an occasional, restricted, water-soaked lesion. No secondary lesions were produced and the bacteria could rarely be isolated from plants 10 days after inoculation. The only host tissues from which the bacteria were consistently recovered were those in the necrotic lesions. Inoculated plants grew very well, as is shown by the fact that their dry weights 2 weeks after inoculation were 90 to 100 per cent that of healthy plants.

The weakly virulent class (index of .20 to .55) included isolates such as No. 165 and No. 172. These isolates invaded a few vascular bundles and produced restricted necrotic lesions. The plants recovered from primary infection, even though the bacteria persisted in the invaded tissues.

The virulent class included the parent culture and a large percentage of the isolates derived from it (Nos. 161, 162, 168, 170, 171, 175, and 177). These isolates produced large necrotic lesions and intense chlorotic streaks. Although many of the invaded leaves died, few of the plants were killed. Secondary invasion occurred and the surviving plants were decidedly dwarfed.

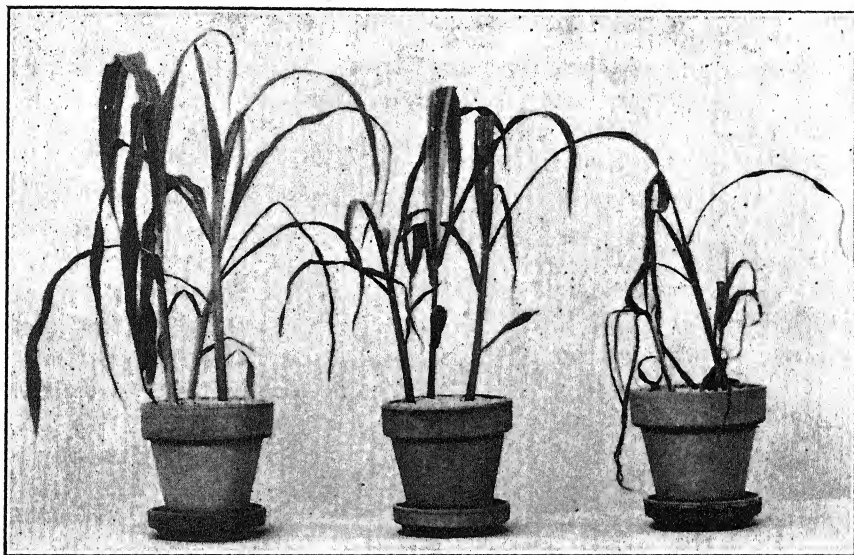
The highly virulent class (index of over .90) included isolates such as Nos. 155, 160, 166, and 176. These isolates produced all the symptoms described for the virulent class and, in addition, caused severe wilting. About half of the plants were completely wilted, and their average dry weight 12 days after inoculation was only 40 to 60 per cent that of the controls. Plants inoculated with slightly virulent, virulent, and highly virulent isolates are illustrated in figure 1.

The isolates placed in each of the 4 classes differed somewhat in pathogenicity. For instance, the virulent class includes isolates Nos. 177, 175, 171, 170, 168, 162, and 161, even though the latter two appear to be less virulent than the others. Further division of these classes was not considered advisable because of the experimental error involved. The infection indexes of most of the virulent isolates varied as much as .10 to .30 in the different experiments.

All the isolates, except Nos. 164 and 173, were similar to the parent culture in colony characteristics. These 2 isolates appeared as white, semi-rough colonies on the dilution plates. When transferred to agar slants, they produced a firm, white, filiform streak. They died after 7 weeks on slants held at room temperature; so they were not tested in experiments B and C. The yellow isolates, with one exception, survived for 3 months under the same conditions. The white isolates proved almost as virulent as the parent culture in the one experiment in which they were tested. They probably arose from the normal yellow type while the bacteria were growing in broth,

since there was no indication that they were present on the agar slant of culture B-11 from which inoculum was transferred. White variants never appeared in visible quantities in the stock culture, nor were they isolated on dilution plates from any of 6 other subcultures seeded from the same slant.

The 3 experiments reported above (Table 1) were repeated in all details with 29 other single-colony isolates from the same subculture. The data obtained confirmed those of the first series of experiments. Although most of the 29 isolates were similar to the parent culture, some were more virulent and some less so. The range in virulence of the isolates was the same as that of the first group tested. The tests show: That subcultures from B-11 con-



Photograph by J. A. Carlile

FIG. 1. Typical wilt symptoms produced on sweet corn 12 days after inoculation by 3 single-colony isolates from a culture of *Phytophthora stewartii* (B-11). The plants on the left were inoculated with a slightly virulent isolate (B11-163); those in the center with a virulent isolate (B11-162); and those on the right with a highly virulent isolate (B11-176).

tained variants that differed in pathogenicity; that the variants were readily isolated by the dilution-plate method; and that the different variants gave about the same results in different inoculation tests extending over a 3-month period.

The stability of 2 variants was tested over a period of a year. A dilution plate was poured with culture B-11, 27 days after it was isolated as a single colony. From this plate a slightly virulent isolate, B-1211, and a highly virulent one, B-1011, were selected for study. These isolates were inoculated into 10-day-old plants from time to time. Culture B-1211 had an index

of .04 in most of the tests and never varied more than from .02 to .08 in different tests. There was no appreciable change in the virulence of this culture. Culture B-1011 usually had an index of about 1.20 (which never varied more than from 1.10 to 1.35) in different tests made during the first 6 months after it was isolated. However, 3 subcultures of B-1011 which were tested during the 9th and 10th months had indexes of .94, .96, and 1.40. Apparently the subcultures seeded from this culture after it had grown on media for more than 6 months were not identical. It is possible that the culture contained variants of different pathogenicities which were transferred in different proportions to the several subcultures.

#### Variants in Different Subcultures from Culture B-11

Single-colony isolates were secured from 7 different subcultures seeded with B-11 at different times over a period of 5 months. The isolates were tested for virulence and arranged in classes according to their infection indexes. A class interval of .10 lesions was used.

The first subculture to be tested was typical of 6 of the 7 subcultures. Each of the 74 single-colony isolates from a dilution plate of this subculture was inoculated into 9 to 12 plants. The infection indexes were computed from readings taken 11 days after inoculation. The frequency distribution of these isolates is presented in figure 2, A. Approximately 75 per cent of the isolates were of intermediate virulence (index 0.70 to 1.10) comparable to that of the parent culture, which had an average index of .90 under the conditions of these experiments. Thirty-three subcultures from the parent culture had indexes that ranged from .75 to 1.07. The single-colony isolates had indexes that ranged from .45 to 1.35; so the range of variability of the isolates was more than twice that of the subcultures. About 25 per cent of the single-colony isolates were less virulent (.45 to .65 lesions) or more virulent (1.10 to 1.35 lesions) than the parent culture.

The 7th subculture yielded a group of isolates with an entirely different range of pathogenicity. The frequency distribution of these isolates is represented by the dotted line in figure 2, A. Most of the isolates had infection indexes similar to that of the parent culture. However, in addition to the weakly virulent isolates present in the first 6 subcultures tested, there were several slightly virulent isolates. These were distinctly less virulent than any of the isolates from the other 6 subcultures. They caused fewer and smaller necrotic lesions than those produced by other isolates.

In the course of other experiments, 19 subcultures of B-11 are tested for variants. Slightly virulent isolates were recovered from only 3 of these subcultures. The infrequent isolation of such slightly virulent strains may be explained in several different ways. Such variants may have existed in all subcultures but escaped detection, since only about 40 colonies from a popu-

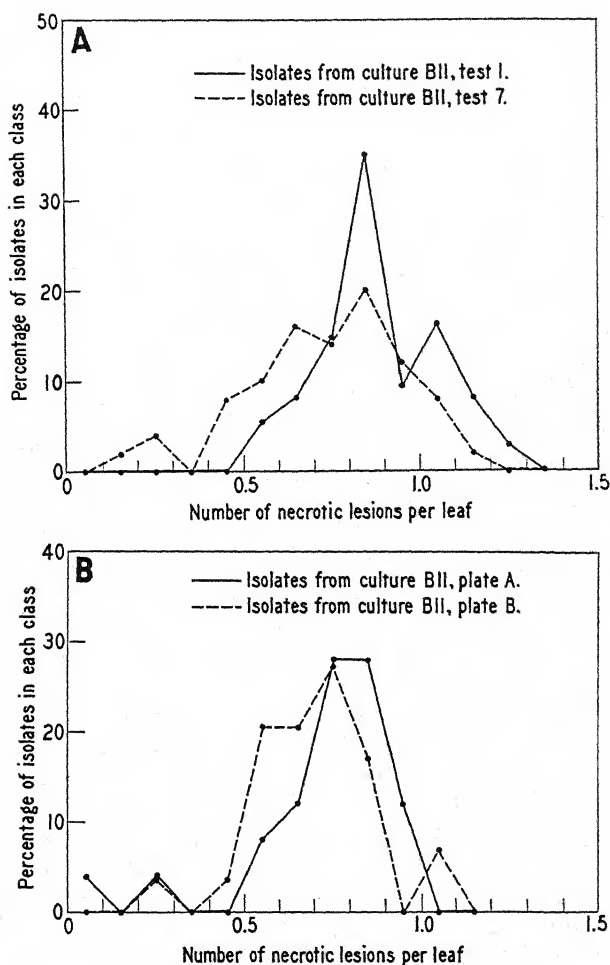


FIG. 2. Frequency distributions of single colony isolates of *Phytomonas stewartii* in pathogenic classes of 0.1 lesions per leaf. A. From pure culture B-11. Test 1 is based upon 74 isolates from one subculture and test 7 upon 48 isolates from another subculture transferred from the same stock culture 7 days later. B. From duplicate dilution plates from same subculture. Twenty-eight and 30 isolates were tested for pathogenicity.

lation of several thousand were tested; the variants may have been present in the stock culture in such small numbers that they were transferred to only an occasional tube of broth; or the variants may have developed in the broth after inoculum was transferred from the stock culture. The following experiment would indicate that they developed after inoculum was transferred to broth.

Two subcultures of B-11, which had been seeded from the same spot in the stock culture in order to secure as nearly identical inoculum as possible,

were tested in duplicate to determine whether one contained a type of variant not present in the other. About 7 hours after the subcultures were seeded, duplicate dilution plates were poured from each. The plates from the first subculture were designated as "A" and "B"; those from the second as "C" and "D". The single-colony isolates from the 4 plates were tested for virulence and classified according to their infection indexes.

The distribution of the isolates from the duplicate plates A and B are presented in figure 2, B. These data show that almost identical variants were present on the 2 dilution plates. Most of the isolates in either plate had an index of about .80. Both plates contained isolates with indexes of less than .50. The only distinct difference in the virulence of the isolates in the 2 plates was that an isolate in plate A with an index of 0.00 had no counterpart in plate B.

The isolates obtained from plates C and D poured with the second subculture had an entirely different range of virulence. The 57 isolates from plate C had infection indexes that ranged from 0.40 to 1.30, while the indexes of the 51 isolates from plate D ranged from 0.50 to 1.10. Neither plates C nor D contained isolates with infection indexes of less than .40, such as those isolated from plates A and B. Since the duplicate tests of both subcultures agree so closely, it would appear that the observed differences indicate a difference in the variant-complex of the 2 subcultures and are not errors due to random sampling. These data substantiate those presented in figure 2, A.

#### Variants from Cultures other than B-11

It was considered of interest to determine whether other cultures of *Phytomonas stewarti* contained variants having different pathogenicities. A culture isolated in New York and another isolated in western Iowa were used. Both cultures produced typical wilt symptoms when inoculated into 10-day-old plants. A subculture from each was dispensed in dilution plates and the single colonies thus obtained were tested for virulence. Both subcultures yielded single-colony isolates that were distinctly less virulent than their respective parent cultures.

#### DISCUSSION

It has been shown that many different types of variants may develop in pure cultures (9, 17), particularly when bacteria are grown in broth media (2, 10, 16). Since the literature on bacterial variation is rather voluminous, the reader is referred to the compilations by Hadley (6) and Brierley (4) for a discussion of the general subject. Very few of the atypical strains reported for the phytopathogenic bacteria have been studied extensively.

However, Ark (1) has shown that cultures of *Erwinia amylovora* (Burr.) Bergey *et al.* differ in virulence and ability to produce variants, and several other investigators (3, 11, 13, 14) have compared the properties of virulent and avirulent cultures of *Phytomonas tumefaciens* (Sm. and Town.) Bergey *et al.* Most of the variants observed in other bacteria have been identified by unusual colony characteristics or physiological abilities. Virulence has, therefore, usually been studied only as it was associated with other characteristics. The observations reported in this paper show that cultures may differ in virulence, even though they are identical in regard to cultural characteristics.

Since strains that differed in virulence were readily isolated from cultures of *Phytomonas stewarti*, the apparent stability of these cultures must depend upon the fact that the variant population remains about constant. Should one strain replace the others, either by growing more rapidly or by chance selection during transfer, the virulence of the entire culture might be changed just as the cultural characteristics of other bacteria have been observed to change as the parent type was replaced by variants (6). Cultures grown on agar slants, however, would not be expected to become avirulent, since the data obtained indicated that the least virulent type of variant developed in nutrient-dextrose broth rather than on agar containing the same nutrients.

Variants that differed in virulence were obtained from all the cultures tested. From this it seems apparent that a corn plant inoculated with a pure culture would be exposed to invasion by a number of pathogenic strains. The strains that develop in the host probably would be the ones best suited to the environment. If selective growth occurs, the variant complex of a culture would be changed by host passage.

#### SUMMARY

The single colonies isolated from dilution plates of virulent cultures of *Phytomonas stewarti* differed in their pathogenic abilities. These variants were classified, according to their ability to produce necrotic lesions and wilting of 10-day-old sweet-corn plants, as: slightly virulent, weakly virulent, virulent, or highly virulent. Slightly virulent isolates occurred infrequently and were isolated from some subcultures but not from others seeded from the same slant.

Two white variants developed during a 7-hour incubation of one subculture. The white colonies obtained were virulent but did not retain their vitality on nutrient-dextrose agar slants so long as the typical yellow cultures.

Cultures isolated from infected corn plants in New Jersey, New York, and Iowa were found to contain variants. These variants were isolated after

the cultures had been run through 5 serial dilutions and single-colony isolations.

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# CRINKLE LEAF, A NEW DISEASE OF COTTON IN LOUISIANA<sup>1</sup>

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## INTRODUCTION

A peculiar disorder of cotton plants, to which the writer has given the name crinkle leaf, has been observed in certain Lintonia and Olivier silt loam soils in Louisiana since 1934, where it was observed in 1934 by H. B. Brown, Agronomist of the Louisiana Experiment Station. These soil types, commonly known as the "bench" or "bluff" soils of Louisiana and Mississippi, are adjacent to the Mississippi River and extend northward from Baton Rouge and beyond Vicksburg, Mississippi. The disease has been observed in 4 separate fields in East Baton Rouge Parish, and in one of them it occurred in a rather severe form in 1937, when the affected plants were discernible within 30 to 40 days from the time of planting and damage to the crop was estimated at about 10 per cent.

The disease has been found in several varieties of upland cotton, including Half and Half, Clevevilt, Express, Stoneville, Dixie-Triumph, Deltapine, and in a sea-island x upland hybrid. Since several varieties are known to be affected, and only in rather definite areas of the field, crinkle leaf would, therefore, appear to be unrelated to hereditary or genetic variations. No evidence for varietal differences in susceptibility or resistance was found.

## SYMPTOMS

The first noticeable feature is the appearance of abnormal leaves. These are puckered, mottled, partially chlorotic, and variously distorted in the early stages, with necrotic lesions subsequently appearing along and between the veins. Later, as the plants approach maturity, they become slightly thickened, brittle, and ragged at the margins (Fig. 1). Fasciation of branches usually occurs, and a peculiar feature occasionally observed is the development of apparently normal branches from the basal nodes of the affected main stem. The involucre bracts, floral buds, flowers, and bolls are reduced in size and are often markedly asymmetric (Fig. 2). The bracts and bolls frequently show chlorophyll deficiency, with the former becoming brownish and necrotic soon after they are formed. The distorted bolls mature very irregularly, some of the carpels opening normally, while others do not open at all. The fiber from such bolls is weak, and the entire lint almost worthless.

In contrast with those of normal plants, longitudinal sections of the terminal branches of affected plants reveal an imperfect or rudimentary devel-

<sup>1</sup> The research here reported was conducted by the Bureau of Plant Industry, U. S. Department of Agriculture, in cooperation with the Louisiana State Agricultural Experiment Station.

opment of the cortical, pith, and vascular tissues. Instead of the stele tissues being sharply defined and whitish in appearance, as in normal plants, they are opaque to light green, and a longitudinal section of the entire branch appears more or less homogenous. Such incomplete development of the stem and branch tissues undoubtedly interferes very greatly with the normal metabolism of the plant.

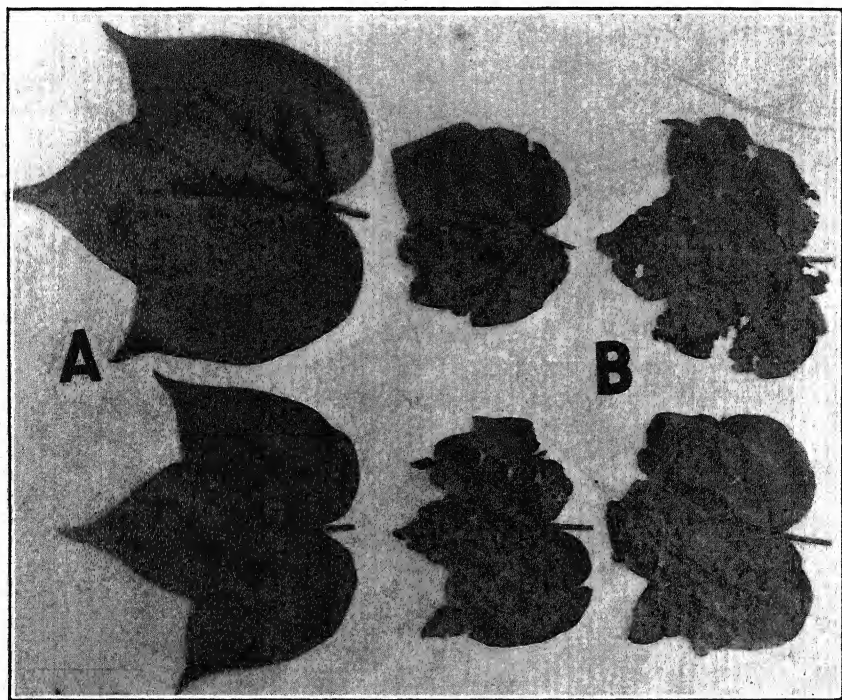


FIG. 1. Leaves of Half and Half cotton plants collected in August, 1936. A. Healthy. B. Affected by crinkle leaf. Note reduction in size and puckered, mottled, and ragged appearance of the leaves.

#### COMPARISON WITH OTHER DISORDERS AND INJURIES CAUSED BY INSECTS

Notwithstanding its similarity in some respects to the "crazy-top" disorder of cotton plants reported from the Southwest, and to that of plants injured by thrips and other insects, crinkle leaf is undoubtedly of a different nature in that it has certain distinctive features not found in cotton plants affected with either of the above mentioned abnormalities. For instance, shedding of flower buds and young bolls, while occurring to some extent, is not characteristically associated with the disease, as is the case with plants affected with crazy top. Neither is there any marked suppression of fruiting branches nor the transformation of fruiting branches into vegetative branches, especially at the top of the plants, as occurs with plants affected

with crazy top. Although fasciation of branches is common and somewhat similar to the injury encountered in young plants that are damaged by thrips, there is no abortion of the terminal bud with resultant sterility, as we often find in plants attacked by thrips.

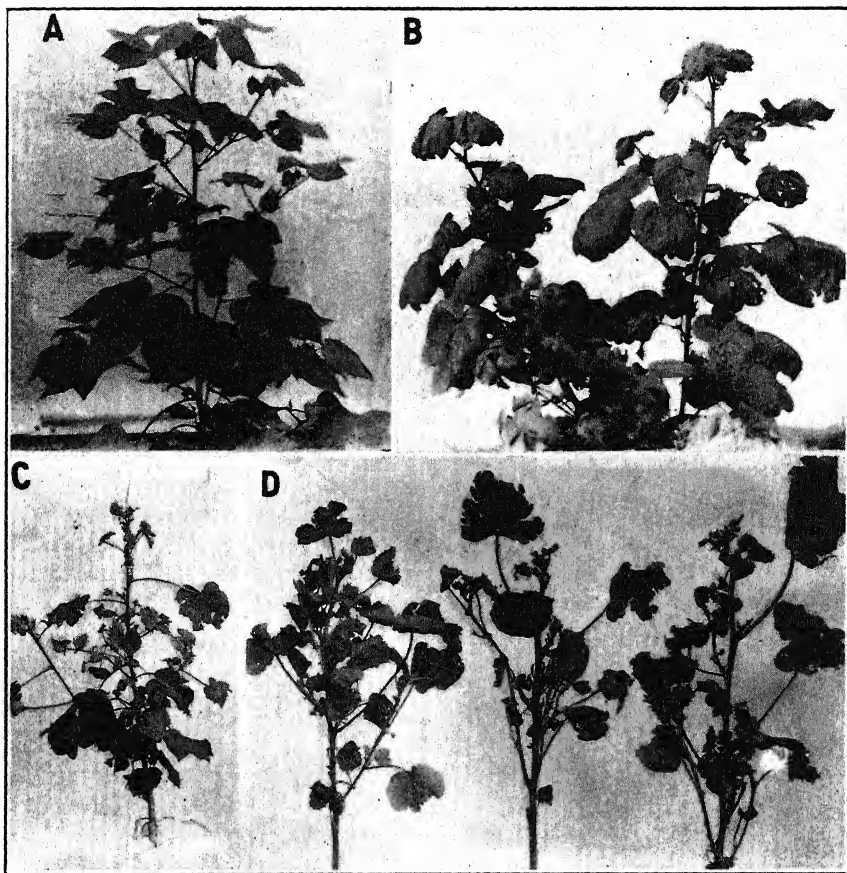


FIG. 2. A-C. Half and Half cotton plants. A. Healthy plant. B and C. Plants showing the effects of "crinkle leaf." Photographed on August 15, 1936. D. Delta-pine cotton plants showing "crinkle leaf" in a severe form. Photographed on June 16, 1937.

Crinkle leaf, however, is similar to crazy top in that it develops quickly and more or less simultaneously in rather definite spots in the field.

#### INOCULATION EXPERIMENTS AND ATTEMPTS TO TRANSMIT THE DISEASE BY GRAFTING AND BUDDING

Certain symptoms of crinkle leaf resemble those of viroses of other plants. Although localization of the disorder and its yearly reappearance in the same areas discouraged the virus concept, it seemed desirable to test this

possibility. Attempts were made during the summer of 1935 to transmit the disease to healthy plants by injecting sap collected from various parts of affected plants. Leaves and branches obtained from typical diseased plants were ground separately and together in a meat grinder and the sap collected in small vials. This was usually employed immediately for inoculation purposes; but, if inoculations could not be made the same day, the extracted sap was placed over night in a low-temperature incubator. Inoculations were made by pricking the leaves, petioles, branches, and main stems of healthy plants with a small needle, the latter being dipped at frequent intervals into the vials containing the sap from the diseased plants. For controls the same procedure was followed, except that sap from healthy plants was used. Eighteen Lone Star plants were inoculated in July, 1935, with sap obtained from six seriously affected Stoneville plants. The inoculated plants, however, grew normally and remained healthy throughout the season. Negative results also were obtained in other similar inoculations during June and July, 1936, when different varieties were used.

In late June and again about the middle of August, attempts were made to transmit the disorder by grafting. Cions were collected from 6 diseased Half and Half and Clewewilt plants, respectively, and top-grafted to the main stems of healthy plants of the same varieties. Similarly, reciprocal grafts also were made with cions from healthy plants. In these tests all of the cions from the affected plants failed to grow, but 4 of those from the healthy plants were successful. They made normal growth and did not develop any symptoms of the disorder. Using the T or shield method of budding, a number of buds from diseased Express plants were placed on healthy Half and Half plants about the middle of August. All of these buds failed to grow. In another experiment buds were collected from healthy plants and placed on the lower and upper portion of the main stem of affected plants. Two of these buds lived and grew normally for the remainder of the season.

In further tests healthy and diseased plants growing side by side in the same row were brought together and inarch grafts made within 4 or 5 inches of the soil line. Although several of these types of grafts were made, only 2 were successful. As soon as the union was strong and sufficiently calloused the plants were tied to supports to avoid breaking, and later, the stems of the healthy plants were cut below the graft and detached from their own root systems. The healthy plants, however, continued to make normal growth throughout the season, despite the fact that their growth was maintained with the rootstocks of affected plants.

#### DISCUSSION AND SUMMARY

A new disease of cotton, described as crinkle leaf, is prevalent in certain soil tracts of Louisiana.

The disease has certain similarities to previously described abnormalities including crazy top, thrip injury, and inherited "round" or "crinkly" leaf. It has, however, definite and distinctive features and is manifestly not hereditary.

Its known distribution is sharply localized to certain *definite* areas in fields where it reappears year after year. Similar symptoms have been obtained in the greenhouse following steam sterilization of Lintonia silt loam.

Crinkle leaf has been observed on several varieties. No varietal differences were found.

Attempts to transmit the disease to healthy plants by several methods described have been unsuccessful.

The nature and cause of the disease is undetermined. Deficiency or excess of some of the minor elements in the soil has been suggested, but is rendered somewhat doubtful by the fact that healthy cions grafted on affected plants have neither developed the disease nor removed it on the stocks.

The disease is being further investigated.

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## ISOLATION OF TOXIC SUBSTANCES FROM THE CULTURE FILTRATES OF TRICHODERMA AND GLIOCLADIUM

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The fungus from whose culture filtrate a crystalline toxic substance has been isolated, appears to be a *Gliocladium*, and is not, as recently reported (8), a *Trichoderma*.<sup>1</sup> Three types of *Trichoderma lignorum* (Tode) Harz were recognized in an earlier paper as O, P, and Q types (7). The symbols A, B, and C are used by Fawcett (1) for the same types: Type A, producing a cocoanut-like odor and causing the typical trichoderma rot of lemons; type B, characterized by the excretion of a bright yellow pigment; and type C, in which were thrown together green *Trichoderma* isolates that belonged neither to type A nor B. Types A and B, as well as most of the cultures of type C, used in the inoculation work with lemons (2), belong to what is commonly considered *Trichoderma lignorum*. One culture regarded in these experiments as belonging to type C (#1927 of the Div. of Plant Path. Citrus Exp. Station, Riverside, Cal.), is the *Gliocladium* mentioned above. The isolates with which other published work has been done (6, 7, 9), are *Trichoderma*.

It has been previously reported (8) that the toxic effects of the used culture solutions of the *Gliocladium* are removed by extraction with chloroform. By the same method, culture filtrates of certain isolates of *Tricho-*

<sup>1</sup> Thanks are due to M. Timonin, New Jersey Agr. Expt. Station, for calling attention to this error, and to Charles Thom, U. S. Dept. Agr., for confirming the identification.

*derma* can be freed also from their fungicidal properties. Likewise, watery solutions of these extracts are toxic to *Rhizoctonia solani* and other fungi at high dilutions. So far, a crystalline substance has not been isolated from these *Trichoderma* extracts.

The most noticeable difference between the toxic effects of the culture filtrates of the two organisms has been found in their relative stability. Culture filtrates of the *Gliocladium*, as well as watery solutions of the crystalline substance extracted from it, may be kept without appreciable loss of activity at room temperature for several days, if an acid reaction is maintained. At alkaline reactions, the solutions lose their toxic effect rapidly, and it can be easily shown that the crystalline toxin is decomposed by alkali, sulphur being split off. For *Trichoderma*, it has been demonstrated previously (7) that, with isolates of the pigmented type, the toxicity of the culture filtrates toward other fungi decreases rapidly, even at very acid reactions, and that with rising alkalinity the rate of decomposition is greatly increased.

The *Gliocladium*, a single-spore culture of which has been used in these studies, had originally been obtained from soil in California. Other isolates of the fungus producing the same crystalline toxic substance have been secured recently from roots of Gerbera plants in New York State.<sup>2</sup> All these organisms appear to belong to the floccose-green series of *Gliocladium* described by Thom (5, pp. 508-509). The description by Gilman and Abbott (3) of *G. fimbriatum* G. and A. fits these organisms fairly well, except that the conidia are somewhat smaller than those reported for *G. fimbriatum*. When grown in various liquid and solid culture media, these *Gliocladium* isolates produce chlamydo-spore-like bodies that are intercalary, and more often terminally disposed on short side branches of the mycelium. The formation of such spores has not been mentioned in the literature.

Horne and Williamson (4) have considered the presence of such chlamydo-spores as a basis of bringing together in the genus *Eidamia* several fungi imperfecti bearing conidial fructifications of unlike types. One of the fungi described by them, *Eidamia virescens* H. and W., obviously corresponds to the type of *Trichoderma*, which produces a cocoanut-like odor in culture. According to Thom (5), this way of classifying the fungi is not justified, and it does not seem to be accepted generally.

A close taxonomic relationship between *Gliocladium* and *Trichoderma* spp. can, therefore, hardly be established on the basis of the formation of chlamydo-spores, especially since they seem to be frequently absent or inconspicuous in both groups. The isolates used are similar in regard to their pathogenic or antagonistic activity towards certain other fungi, when associated with them in culture media. But this is a physiological character that they have in common also with many unrelated organisms.

<sup>2</sup> The writer is indebted, for these cultures, to F. A. Haasis, of Cornell University.

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## THE ANATOMY OF A BLACK ZONE CAUSED BY XYLARIA POLYMORPHA

M. T. HILBORN

(Accepted for publication Aug. 20, 1937)

In 1933 the author prepared microscopic sections of a black zone found in a stump of red maple, *Acer rubrum* L. All attempts to isolate the fungus or fungi responsible for the zone were unsuccessful. Shortly after these sections had been examined, Campbell (2, 3, 4) published the first of his studies on zone lines in plant tissue. As some of the author's conclusions did not agree with Campbell's published results on the development of black zones, observations were continued. In July, 1937, fruiting bodies of *Xylaria polymorpha* (Pers.) Grev. appeared on the wood of this stump, and a similar black zone was found in sections of the wood near these fruiting bodies. Microscopic examination of this zone disclosed the same characters as those observed in 1933.

According to Campbell (2) the black zone is characterized by the formation of bladder cells (Fig. 1, C) that form a dense shell inclosing a mass of the host tissue. This tissue and its inclosing layer of bladder cells Campbell (3) terms a "pseudosclerotium." It is different from a true sclerotium in that it consists partly of host cells and is perennial, and it differs in plasticity. He also states (2) that the zone line is not progressive. Hiley (6, pp. 155-158) in figuring and describing the zone line caused by *Armillaria mellea* in "common larch" states that the zone line moved progressively in the host tissue, thus substantiating a previous statement by Hartig (5, p. 59).

Campbell, in objecting to the progressive-movement concept, says that it was not apparent in his sections or in sections prepared from Hartig's ma-



terial in the Museum of the Royal Botanic Garden at Edinburgh, Scotland. However, the writer is inclined to agree with Hiley. There is a remarkable similarity between figure 1, D, and the illustration given by Hiley (6) on

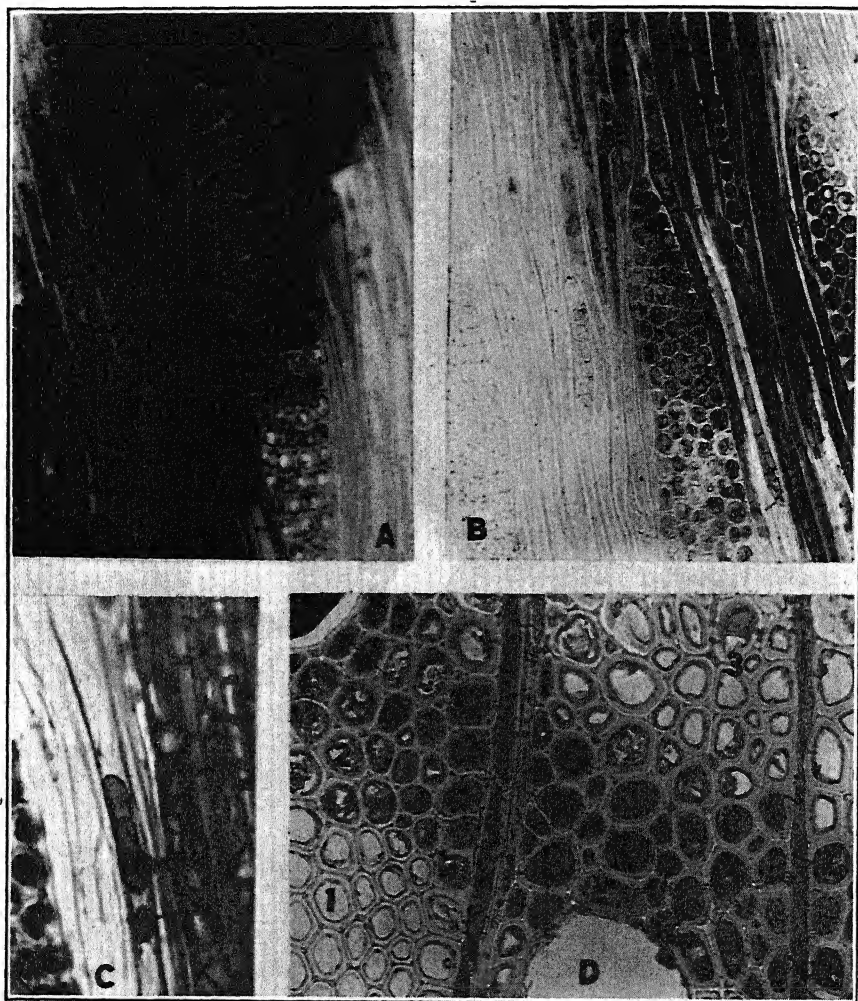


FIG. 1. A. Tangential section of a black zone as it appears when sectioned by the microtome.  $\times 350$ . B. Tangential section after treatment with chlorine gas.  $\times 350$ . C. Bladder cells in a tangential section.  $\times 800$ . D. Cross section of a black zone.  $\times 500$ .

page 157. As is shown in figure 1, D and B, the black zone consists of brown, sometimes almost black, bladder cells, crowded together and occupying the lumina of the fibers and the wood ray cells. There appear to be several stages in the formation of this black zone, the details of which are



evident after the sections have been exposed to chlorine gas. At first numerous hyaline hyphae penetrate the tissue (Fig. 1, D-1). They later swell to enormous size and become closely septate (Fig. 1, B). A hypha may be traced in the section. At one edge of the zone the hypha appears perfectly normal, at another point, farther in, it begins to form a bladder cell; and, still farther in, the bladder cell apparently breaks down and exudes a pigment that in turn, stains the surrounding tissue (Fig. 1, A and 1, D-2). Finally, the pigment disappears leaving only the broken bladder cells just outside the other edge of the zone and these in turn disappear (Fig. 1, D-3). It thus seems that a progressive movement of the zone line does occur, at least in nature. No observations were made on material in artificial culture.

It is apparent, however, that the bladder cells do not represent "a more active stage in the metabolism of the fungus," as suggested by Hiley (6) and objected to by Campbell (2). The theory offered by Campbell (3, 4), that the zone line is a "pseudosclerotium" seems logical.

The author's inability to culture the fungus from the zone line in 1933 raised the question as to whether or not the organism was alive. Brooks and Brenchley (1) found that when a barrier of gum inclosed the infected tissues, the fungus died within a year. This they attributed to staling and its associated phenomena. In July, 1937, no difficulty was experienced in securing cultures of the fungus from wood bearing fruiting bodies. Possibly the previous failure can be attributed to staling.

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## PHYTOPATHOLOGICAL NOTES

### *A Severe Case of Rhizoctonia Root Rot of Sugar Beets After Potatoes.*<sup>1</sup>

The writer is not aware of any recorded observations that potato isolates of *Rhizoctonia solani* Kühn can cause the late canker root rot of sugar beets. In 1935, at Kanawha, Iowa, there was a severe case of late canker root rot of sugar beets grown on a piece of ground devoted to potatoes in 1934. Adjoining the potatoes there had been a field of barley. The beets covered all of the potato ground and some of the barley field. On the latter area there was scarcely any rot (Table 1). The author's attention was attracted because

TABLE 1.—Number of healthy and late root-rotted sugar beets per 50-ft. row in 2 adjacent areas that had been devoted to potatoes or barley the previous year

Sample row	Potato area		Barley area	
	Healthy	Rotted	Healthy	Rotted
1 .....	20	11	30	2
2 .....	15	18	31	0
3 .....	23	15	36	0
4 .....	9	19	25	0
Ave. ....	16½	15½	30½	½

the rotten beets in the potato area closely resembled those that had been rotted by *Rhizoctonia* in inoculation experiments the previous season (Fig. 1).

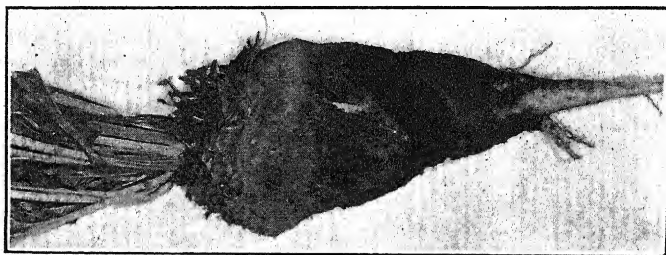


FIG. 1. Beet rotted in 1934 after inoculation with *Rhizoctonia*. Many of the beets in the potato area looked like the one shown.

The only fungus recovered from the rotted beets was *Rhizoctonia*. Sample rows for counts of the 2 areas were taken as closely together as possible without leaving doubt as to their correct location. Nearly 50 per cent of the stand rotted on the potato ground, while only 1.6 per cent rotted on the land used for barley in 1934.—W. F. BUCHHOLTZ, Botany and Plant Pathology Section, Iowa State College, Ames, Iowa.

<sup>1</sup> Journal Paper No. J491 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 432.

*The Genus Phytomonas.*—The use of the genus name *Phytomonas* by the committee of the Society of American Bacteriologists in Bergey's Manual of Determinative Bacteriology, 1923, and the adoption of this terminology by many plant pathologists is unfortunate, as this genus name was used by Donovan in 1909 for a group of flagellate protozoans in the latex of plants.

In recent publications Gerold Stahel has used *Phytomonas leptovosorum* as the name for the flagellate protozoan occurring in the sieve-tubes of diseased Liberian coffee trees in Surinam. This is the proper use of the genus name *Phytomonas*.

In this connection the following citations and quotations will be of interest to plant pathologists:

Lafont, A. Sur la présence d'un parasite de la classe des Flagellés dans le latex de l'*Euphorbia pilulifera*. Comptes Rendus Soc. Biol. 66: 1011-1013, 1909. P. 1012 "En somme, ce Flagellé présente tous les caractères du genre *Leptomonas* (*Herpetomonas* au sens de certains auteurs).—Nous le désignerons sous le nom de *Leptomonas Davidi*."

Donovan, C. Kale-azar in Madras, especially with regard to its connection with the dog and the bug (*Conorrhinus*). Lancet 177: 1495-1496. 1909.

"Lafont has recently found herpetomonas (leptomonas) in the latex of *Euphorbia pilulifera* in Mauritius. I have confirmed his find and have discovered these flagellates, small narrow forms, in the latex of the same plants growing in Madras. The organisms differ from the known flagellates parasitic on animals and will doubtless be placed in a new genus, for which I suggest the name of *Phytomonas*."

Wenyon, C. M. Observations on the intestinal protozoa of three Egyptian lizards, with a note on a cell-invading fungus. Parasitology, 12: 350-365, 1920. P. 356. "For these flagellates we may employ the name *Phytomonas*, first suggested by Donovan (Lancet, 1909), the type species being *Phytomonas davidi* (Lafont, 1909)."

Bergey's Manual of Determinative Bacteriology, p. 174. Genus VIII, *Phytomonas* gen. nov. 1923 (36 species of bacteria are listed as parasitic on plants.)

Second International Congress for Microbiology, London, 1936. Report of Proceedings: London. 1937. P. 29 under resolutions of the nomenclature committee passed by the Plenary Congress.

1. "(e) It was agreed that the genus *Bacillus* should be so defined as to exclude bacterial species which do not produce endospores."

2. "(a) Generic homonyms are not permitted in the group *Protista*.

(b) It is advisable to avoid homonymy amongst *Protista* on the one hand, plants or animals (Metazoa) on the other."

Review of Applied Mycology 16: 482, 1937.

"In view of this decision [by the London Congress 1936] the use of the name *Bacillus* for bacterial species not producing endospores is clearly invalid, and consequently it cannot be retained by plant pathologists for non-sporing bacteria motile by means of peritrichiate flagella."

The generic name *Erwinia* for plant pathogenic rods with peritrichiate flagella has already been quite generally adopted and will no doubt be still more widely used, now that the name *Bacillus* has been definitely declared invalid.

It is apparent from the above citations that protozoologists have prior claim to the genus name *Phytomonas* and that resolution 2 (a and b), adopted by the London Congress in 1936, excludes *Phytomonas* for plant pathogenic bacteria with polar flagella or none.

For the present at least there are two alternatives for plant pathologists

1. To follow Migula's system and use *Pseudomonas* for polar flagellate and *Bacterium* for nonmotile rods.
2. To follow Smith and use *Bacterium* for polar flagellate and *Aplanobacter* for nonmotile plant pathogenic bacteria.

Our present limited knowledge of the group characteristics of polar flagellate and nonmotile bacterial plant pathogens makes it advisable, however, to retain these two groups in separate genera.—CHARLOTTE ELLIOTT, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

*Method of Isolating Single Hyphal Tips of Actinomyces.*—*Actinomyces* is a very minute organism, and many strains so closely resemble one another that it is often very difficult to differentiate them, even microscopically, one from the other. Consequently, in the study of *Actinomyces*, it is especially important to know that an investigation is being conducted with pure cultures. The author, in his work on potato scab,<sup>1</sup> made hyphal-tip isolations before the physiology of the organism was studied.

The following method was found to be satisfactory in isolating pure strains of *Actinomyces* to be studied. Because of the minuteness of the spores and the absence of a micromanipulator, isolations of *Actinomyces* were made by cutting off the tips of the aerial hyphae. When a culture of *Actinomyces* is grown in a Petri dish and observed under a microscope, one sees that the mycelium growing in the medium sends out strands of aerial hyphae that often stand in a vertical position. If a high dilution of the organism is used, the aerial hyphae frequently grow as separate threads on the agar. The technique used in cutting off the tips of aerial hyphae was based largely on the methods described by Edgerton<sup>2</sup> and Brown.<sup>3</sup>

<sup>1</sup> Afanasiev, M. M. Comparative physiology of *Actinomyces* in relation to potato scab. Nebraska Agr. Expt. Sta. Res. Bull. 92. (In press.)

<sup>2</sup> Edgerton, C. W. A method of picking up single spores. *Phytopath.* 4: 115-117. 1914.

<sup>3</sup> Brown, W. Two mycological methods. *Ann. Bot.* 38: 401-404. 1924.

A microscope with a substage condenser was used. The diaphragm under this condenser was swung out and to it was attached, by means of "vultex" vulcanized rubber, a large cork with an ordinary rubber tube clamp, and this, in turn, was fixed to the cork with a rubber band.

Very thin glass needles were made with slightly bent, pointed ends. One end of a glass needle was fixed horizontally in the clamp above the stage with the fine point reaching to the field of the microscope. The mechanism that regulated the substage of the microscope gave horizontal and vertical movement to the glass needle.

Young cultures on clear albumin agar in Petri dishes were observed under the microscope and a portion of the agar supporting sufficiently separated aerial hyphae was placed on a sterile microscope slide and again examined with a low-power objective.

When a hypha was found sufficiently separated from others, its tip was lightly touched with the point of the needle which, previously had been dipped in a very dilute, sterile solution of sucrose. This resulted in the tip of the hypha sticking to the needle and breaking away as the condenser was slightly raised and the needle carefully withdrawn. The point of the needle was then carefully examined to determine if a single tip of aerial hypha had been isolated. The point with the single hyphal tip on it was then immersed and broken off in sterile albumin agar in a Petri dish.

After 3 to 4 days, it was possible to observe with a microscope the beginning of growth from this isolated piece of aerial hypha. When growth was sufficient, this culture was transferred to a test tube containing 2 per cent potato dextrose agar (20 per cent potato, 2 per cent dextrose, and 2 per cent agar). Thus it was possible to isolate single hyphal cultures of 24 *Actinomyces* used in the above-mentioned investigation.—M. M. AFANASIEV, Department of Plant Pathology, College of Agriculture, Lincoln, Nebraska.

## BOOK REVIEWS

RIKER, A. J., AND RIKER, REGINA S. *Introduction to research on plant diseases.* 117 pp. Illus. 1936. (Published by the authors, University of Wisconsin. \$2.65.)

This is an excellent book, both in form and content. It is planographed two columns per page; index, three columns. The sheets are  $8\frac{1}{2} \times 11$  inches, with press-board covers, all bound with double wire-ring binding in such a way as to open flat, which is a very real convenience in use.

The book is much more than a manual. It is, as the title states, an "introduction to research on plant diseases"; and also, as further elaborated in the subtitle, it is "a guide to the principles and practice for studying various plant-disease problems."

In the introductory chapter, entitled "preliminary considerations," are clearly discussed, the fundamentals of (1) the scientific method, (2) research in plant pathology and its relation to other subjects, and (3) cooperative research. This can be read with profit by any plant pathologist or other scientist, young or old, or by any administrator of research projects.

The body of the text is given in 11 chapters with the following well-chosen, largely self-explanatory headings: Chapter I, Foundation of a research problem; Chapter II, General laboratory equipment; Chapter III, Culture media; Chapter IV, Certain physical-chemical measurements; Chapter V, Isolation, culture, and inoculation; Chapter VI, Virus diseases; Chapter VII, Pathological histology; Chapter VIII, Epidemiology, environment, and control; Chapter IX, Statistical analyses; Chapter X, Records and manuscripts; and Chapter XI, Laboratory exercise topics. The subject matter of each of these chapters is admirably presented, and given in sufficient detail, together with illustrations, so that the material can be of very specific help for anyone dealing with these problems. At the end of each chapter are well chosen literature citations bearing on the subject matter of the chapter.

The last chapter (Chapter XI), entitled "Laboratory exercise topics," covers much more than its title implies. The chapter is divided into the following three sections: The research attitude; topics according to chapters; and topics according to representative diseases. Each of these has a large number of specific topics, through which both the theoretical and practical matters previously presented may be tried out and put into practice.

Following the last chapter is a one-page appendix, which has two divisions: A set of conversion tables, and a set of general laboratory rules. Both of these are very useful. While many of the rules seem very simple, yet it is well to have them written out for all of us, young and old, to read over and strive to live up to. An excellent index, a couple of very suggestive blank forms, and several blank pages for notes, complete the volume.

While the volume is a veritable mine of information both as to details and sources, yet I am inclined to think that its most valuable contribution is the perspective that it gives and the painstaking organization it suggests, from the very first attack on a problem to the last stroke on the finished manuscript.

One cannot but wish, however, that at least two more chapters had been added, one on nomenclatorial matters and another on physiologic races. Nearly every plant pathologist must deal with these subjects to a greater or lesser extent; and it would have rounded out the volume had they been included. Possibly we may hope for these in future revisions. A. G. JOHNSON, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

BRAUN, PROF. DR. HANS. *Pflanzenhygiene.* 98 pp. Paul Parey, Berlin. 1937.

The booklet on plant hygiene written by Professor Braun brings together the practical applications and ideas relative to prevention of disease in plants by hygienic methods or those practices that deal with the production of a healthy plant that is better able to counteract the attacks of plant pathogens. The importance of considering the healthy plant before it becomes diseased is stressed as the prevention of disease often is considered to be cheaper and easier than the curing of disease.

Professor Braun has systematically dealt with each phase and question of plant hygiene as it is related to general agriculture, gardening, and forestry. In developing the topic the writer has divided the booklet into 3 major parts dealing with the culture, disinfection, and quarantine phases of the problem.

The discussion of general culture of plants is divided into 3 parts; i.e., consideration of the locality or place of planting; an improvement of the locality or place of planting; and the plant as a direct subject for hygienic cultural measures. Under locality, the importance of growing plants in their ecological optimum environment is stressed. Individual local factors, such as climate, soil, and site, are discussed. It is pointed out that planting of species and varieties should be made according to their climatic requirements. Such factors as adaptability to varying degrees of humidity, frost resistance, and winter cold are cited. If plants are grown out of their environment they may be more subject to attack by parasitic fungi. The importance of selection of the proper soil for each particular crop is dealt with, for, as stated, the soil may have a direct bearing on the development of the parasite, as well as that of the plant. Mention is made of danger from hail, early frost, temperature, local humidity, air movement, shade, and wind in localized areas. The necessity for selection to avoid these conditions for certain crops is pointed out.

The question of possible improvement of the locality or place of planting is discussed under possible methods of changing, directly or indirectly, the effects of meteorological conditions. Particular reference to temperature, protection from frost, and methods of bettering the soil conditions, primarily from a water-holding standpoint, is considered. Importance of cultivation from a standpoint of aeration is taken up. Lastly, the effect and need of fertilization are discussed. The possible lack of lesser elements and their relationship to disease and the known relationships of nitrogen, phosphorus and potassium to aid in inducing or warding off disease are mentioned. It is finally pointed out that the correct handling of fertilizers aids in the production of healthy plants that may ward off disease, but that the inherent genetic factors of the different plant types are much more important from this standpoint.

Under the discussion of the plant as a direct subject for hygienic cultural treatment, the following measures are mentioned: rotation, variety selection, seed selection, date and depth of seeding, and spacing. It is pointed out that the proper management of the above factors will aid in the production of healthy plants that may resist diseases. The importance of the planting of resistant varieties on infested soil is stressed.

The second main division of the booklet considers disinfection. The subject is discussed under soil disinfection and seed or plant disinfection. Three methods of soil treatment are pointed out, these being biological, physical, and chemical. From a biological standpoint the length of life of parasites in the soil in relation to rotation is discussed. The physical disinfectant methods mentioned are the use of hot water and steam to sterilize soil. Formalin, carbon bisulphide, paradichloral benzene, potassium sulpho-carbonate, mercuric chloride, Uspulun, carbolinum, gasoline, and petroleum are discussed under chemical soil disinfectants.

Disinfectant treatments of the seed and plant are elaborated upon under physical treatments, such as hot water and dry heat, and chemical methods, such as chemical dips and the use of disinfectant dusts. Formalin, mercuric chloride, and the organic mercury compounds are mentioned. The difference in tolerance, especially by the different vegetable seeds, is pointed out. Finally, the use of cyanids and carbon bisulphide as gas disinfectants is discussed.

The third and last main division of the booklet takes up plant quarantine. A résumé of the subject with particular reference to the importance of a quarantine for Germany is given. Examples are cited of the introduction or spread of various insects and fungi into and through Europe, including the potato beetle, *Phylloxera vastatrix*, grape mildew, hop mildew, Graphium elm disease, and others. The methods of quarantine are further discussed under border and regional quarantine.

The booklet is clearly and simply written. It apparently has been prepared for student use in the universities, but it should be useful to all interested in general agriculture, gardening, and forestry practices.—OTTO A. REINKING, N. Y. State Agr. Exp. Station, Geneva, New York.

## NOTE

The American type-culture collection of fungi and bacteria, formerly located at the McCormick Institute in Chicago, Ill., has recently been moved to Washington, D. C. and has been installed in the Georgetown University Medical School Building.

The collection will be in charge of Dr. Mario Mollari, Professor of Bacteriology at the University, with an assistant, Dr. Oswald A. Bushnell. Any cultures of new or interesting species of organisms of these groups will be greatly appreciated. A catalogue of the collection is now being prepared. Contributions should be sent as soon as convenient in order that they may be incorporated in the new list.

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MEMBER OF THE EXECUTIVE COMMITTEE FROM

THE AMERICAN PHYTOPATHOLOGICAL SOCIETY.